U.S. DEPARTMENT OF COMMERCE
National Technical Information Service

PB-286 830

Fate and Impact of Pentachlorophenol in a Freshwater Ecosystem

University of Southern Mississippi, Hattiesburg

Prepared for

Environmental Research Lab, Athens, GA

Jul 78

United States
Environmental Protection
Agency

Environmental Research
Laboratory
Athens GA 30605

EPA-600/3-78-063
July 1978

Research and Development

PB 286830

EPA

Fate and Impact of Pentachlorophenol in a Freshwater Ecosystem

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U. S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA. 22161

RESEARCH REPORTING SERIES

Research reports of the Office of Research and Development, U.S. Environmental Protection Agency, have been grouped into nine series. These nine broad categories were established to facilitate further development and application of environmental technology. Elimination of traditional grouping was consciously planned to foster technology transfer and a maximum interface in related fields. The nine series are:

1. Environmental Health Effects Research
2. Environmental Protection Technology
3. Ecological Research
4. Environmental Monitoring
5. Socioeconomic Environmental Studies
6. Scientific and Technical Assessment Reports (STAR)
7. Interagency Energy-Environment Research and Development
8. "Special" Reports
9. Miscellaneous Reports

This report has been assigned to the ECOLOGICAL RESEARCH series. This series describes research on the effects of pollution on humans, plant and animal species, and materials. Problems are assessed for their long- and short-term influences. Investigations include formation, transport, and pathway studies to determine the fate of pollutants and their effects. This work provides the technical basis for setting standards to minimize undesirable changes in living organisms in the aquatic, terrestrial, and atmospheric environments.

This document is available to the public through the National Technical Information Service, Springfield, Virginia 22161.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA-600/3-78-063	2.
3. RECIPIENT'S ACCESSION NO. PB286830	
4. TITLE AND SUBTITLE Fate and Impact of Pentachlorophenol in a Freshwater Ecosystem	5. REPORT DATE July 1978 issuing date
	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) Richard H. Pierce, Jr.	8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Institute of Environmental Science University of Southern Mississippi Hattiesburg, MS 39401	10. PROGRAM ELEMENT NO. 1HE775
	11. CONTRACT/GRANT NO. R-803820
12. SPONSORING AGENCY NAME AND ADDRESS Environmental Research Laboratory - ATHENS, GA Office of Research and Development U.S. Environmental Protection Agency Athens, GA 30605	13. TYPE OF REPORT AND PERIOD COVERED Final 7/75-11/77
	14. SPONSORING AGENCY CODE EPA/600/01
15. SUPPLEMENTARY NOTES	

16. ABSTRACT

This investigation was undertaken to determine the fate of pentachlorophenol (PCP) that caused extensive fish kills in a freshwater lake in December 1974 and again in December 1976. The kills resulted from the accidental release of wood-treating wastes containing PCP in fuel oil. Food chain relationships were investigated in the lake and the accumulation and elimination of sublethal concentrations of dissolved PCP was studied under laboratory conditions for the bluegill (Lepomis macrochirus). The highest concentrations of PCP in fish were observed in the bile followed by liver, gills, and muscle.

Lake sediment and leaf litter contained high concentrations of PCP throughout the two-year study. Studies of leaf litter from the contaminated water shed area showed it to be a source for chronic pollution of the aquatic ecosystem. The major degradation products observed were pentachloroanisole (PCP-OCH_3) and the 2,3,5,6- and 2,3,4,5-tetrachlorophenol (TCP) isomers. These products were found to persist in sediment and fish along with PCP. The methyl ethers (anisoles) of both TCP isomers and the 2,3,4,6-TCP isomer were observed in some samples but the small amounts were difficult to quantitate.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Phenol Toxicology Degradation Water pollution	Chlorinated phenols Bioaccumulation Environmental fate Aquatic degradation Environmental persistence	06F 06T 68D

18. DISTRIBUTION STATEMENT Release to Public	19. SECURITY CLASS *(This Report)* Unclassified	21. NO. OF PAGES 74
	20. SECURITY CLASS *(This page)* Unclassified	22. PRICE MF A01 PC A04

* U.S. GOVERNMENT PRINTING OFFICE: 1978—757-140/1395

EPA-600/3-78-063
July 1978

FATE AND IMPACT OF PENTACHLOROPHENOL IN A FRESHWATER ECOSYSTEM

by

Richard H. Pierce, Jr.
Institute of Environmental Science
University of Southern Mississippi
Hattiesburg, Mississippi 39401

Grant Number R-803-82-0010

Project Officer

N. Lee Wolfe
Environmental Processes Branch
Environmental Research Laboratory
Athens, Georgia 30605

ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
ATHENS, GEORGIA 30605

DISCLAIMER

This report has been reviewed by the Environmental Research Laboratory, U.S. Environmental Protection Agency, Athens, Georgia, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the U.S. Environmental Protection Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

FOREWORD

Environmental protection efforts are increasingly directed towards prevention of adverse health and ecological effects associated with specific compounds of natural or human origin. As part of this Laboratory's research on the occurrence, movement, transformation, impact, and control of environmental contaminants, the Environmental Processes Branch studies the microbiological, chemical, and physico-chemical processes that control the transport, transformation and impact of pollutants in soil and water.

Human illness and death have occurred from exposure to pentachlorophenol (PCP) in industrial and agricultural applications. In 1974 and 1975, accidental release of wood-treatment wastes containing PCP in fuel oil caused extensive fish kills in a freshwater lake in Mississippi. The study reported here examines the persistence and distribution of PCP and PCP-degradation products in this lake to provide information on the effects of PCP contamination of aquatic systems.

David W. Duttweiler
Director
Environmental Research Laboratory
Athens, Georgia

ABSTRACT

This investigation was undertaken to determine the fate of pentachlorophenol (PCP) that caused extensive fish kills in a freshwater lake in December 1974 and again in December 1976. The kills resulted from the accidental release of wood-treating wastes containing PCP in fuel oil. Samples of water, suspended particulates, sediment, leaf litter and fish were collected from February 1975 through April 1977 in an attempt to determine the persistence and distribution of PCP and PCP-degradation products in the aquatic environment.

Food chain relationships were investigated in the lake and the accumulation and elimination of sublethal concentrations of dissolved PCP were studied under laboratory conditions for the bluegill (Lepomis macrochirus). Also investigated were the solubility of PCP in water at various pH levels and the release of PCP from contaminated watershed material.

Concentrations of PCP well above background levels were found in the water and in fish for over six months following the first spill with another increase observed fourteen months after the spill (February 1976) following a period of heavy rain. After the second spill, high PCP concentrations were observed in samples collected in January 1977, and samples collected in April 1977 showed that PCP still remained in water and fish four months after the spill. The highest concentrations in fish were observed in the bile followed by liver, gills, and muscle. Lake sediment and leaf litter contained high concentrations of PCP throughout the two-year study. Studies of leaf litter from the contaminated watershed area showed it to be a source for chronic pollution of the aquatic ecosystem.

The major degradation products observed were pentachloroanisole ($PCP\text{-}OCH_3$) and the 2,3,5,6- and 2,3,4,5-tetrachlorophenol (TCP) isomers. These products were found to persist in sediment and fish along with PCP. The methyl ethers (anisoles) of both TCP isomers and 2,3,4,6-TCP isomer were observed in some samples but the small amounts were difficult to quantitate. The results suggested that $PCP\text{-}OCH_3$ was formed within the aquatic environment, whereas much of the TCP appeared to have been formed before entering the lake, perhaps by photoreduction in the fuel oil solution.

At the pH of the lake water, PCP existed primarily as the phenate anion and the distribution of PCP throughout the water column was enhanced by the solubility. The acute toxicity to fish observed immediately after each spill occurred by uptake of the phenate anion dissolved in water. Food chain relationships within the lake showed that game fish populations depended ultimately upon benthic organisms as a food source. The accumulation of PCP in sediments, therefore, provided a source for chronic pollution of fish caught for human consumption.

This report was submitted in fulfillment of grant no. R-803-82-0010 by the University of Southern Mississippi, Institute of Environmental Science under the sponsorship of the U.S. Environmental Protection Agency. This report covers a period from January 1975 to April 1977 and the work was completed as of August 31, 1977.

CONTENTS

Foreword iii
Abstract iv
Figures. viii
Tables ix
Acknowledgement. xi

1. Introduction. 1
2. Conclusions 5
3. Recommendations 7
4. Materials and Methods 9
    Environmental samples. 9
    PCP release from contaminated leaf litter. 13
    Solubility of PCP in water 13
    Accumulation and elimination of PCP in fish. 16
    Food chain study 17
5. Results and Discussion. 19
    Environmental samples. 19
    PCP release from contaminated leaf litter. 34
    Solubility of PCP in water 36
    Accumulation and elimination of PCP in fish. 42
    Food chain study 47

References 56
Appendix 59

Preceding page blank

FIGURES

Number		Page
1	Sample sites in contaminated stream and lake	2
2	Extraction of PCP and PCP-degradation products from environmental samples.	12
3	Titration of PCP in aqueous solution	38
4	Ultraviolet spectra of PCP and SPCP in 0.1 M buffer.	39
5	Accumulation of PCP in tissue during exposure to 0.1 ppm PCP	44
6	Elimination of PCP from Group 1 (after 4 days exposure).	45
7	Elimination of PCP from Group II (after 16 days exposure).	46

TABLES

Number		Page
1	PCP and PCP-Degradation Products in Control Samples	20
2	PCP in Water Samples Collected February 1975 - May 1976	21
3	PCP in Water Samples Collected August 1976 - April 1977	22
4	PCP in Sediment Samples Collected February 1975 - May 1976.	24
5	PCP in Sediment Samples Collected August 1976 - April 1977.	25
6	PCP in Fish Collected February 1975 - May 1976.	25
7	PCP in Fish Collected August 1976 - April 1977.	26
8	PCP in Leaf Litter.	27
9	PCP and Degradation Products in Technical Grade PCP, Industrial Waste Holding Pond and Oil Slick from Stream.	29
10	PCP-Degradation Products in Lake Water (Dissolved) Collected August 1976 - April 1977.	30
11	PCP-Degradation Products in Lake Sediment Collected August 1976 - April 1977.	31
12	PCP-Degradation Products in Fish Collected August and October 1976.	32
13	PCP-Degradation Products in Fish Collected January and April 1977	33
14	PCP-Degradation Products in Leaf Litter Collected August 1976 - April 1977.	35
15	PCP Release from Contaminated Leaf Litter	36
16	Dissociation of PCP in Water.	37
17	Spectrophotometric Characteristics in the Ultraviolet of PCP and SPCP.	40
18	Solubility of 26°C of PCP and SPCP at Three pH Values Using Two Analytical Methods.	41

19 LC-50 of PCP to Lepomis machrochirus 43

20 Accumulation of PCP in Fish Tissue after Exposure to 0.1 mg PCP/l Water. 43

21 Elimination of PCP from Group II Fish, Placed in a Clean Environment after Sixteen Days Exposure to PCP-Contaminated Water 47

22 Benthic Organisms Collected During August and October 1976 49

23 Benthic Organisms Collected During January and April 1977. 50

24 Stomach Contents of Sunfish Collected from August 1976 - April 1977. 52

25 Stomach Contents of Catfish Collected from August 1976 - April 1977. 53

26 Stomach Contents of Non-game Fish Collected from August 1976 - April 1977 54

A-1 Physical Parameters from Water Sampling Sites from February 1975 to May 1976. 59

A-2 Physical Parameters from Water Sampling Sites from August 1976 to April 1977. 61

ACKNOWLEDGEMENTS

Special acknowledgement is given to Dr. C. R. Brent, Institute of Environmental Science, and Dr. H. P. Williams, Department of Chemistry, University of Southern Mississippi, for their involvement as coinvestigators during the first year grant project and for their continued assistance throughout the project period. Several people were involved with various aspects of the investigation. The major contributers were: Dr. D. M. Victor, postdoctoral research fellow, Institute of Environmental Science, analysis of PCP and degradation products in environmental samples; Dr. M. S. Torrey, chemical consultant, solubility and pH studies; S. R. Reeves, graduate assistant in the Department of Chemistry, analysis of environmental samples; G. W. Pruitt, graduate assistant in the Department of Biology, study of the accumulation and elimination of PCP in bluegills; and T. C. Modde, graduate assistant in the Department of Biology, study of food chain relationships in the contaminated lake.

Fish used for the accumulation and elimination studies were supplied by the Mississippi Game and Fish Commission Lyman Fish Hatchery, Lyman, Mississippi. The Department of Biology, University of Southern Mississippi, provided boats and equipment for the collection of environmental samples and the gas chromatograph with accessories was supplied by the Department of Chemistry.

The cooperation of the project director, Dr. N. L. Wolfe, of the U.S. Environmental Protection Agency's Environmental Research Laboratory, Athens, Georgia, throughout the investigation and in obtaining GC-MS analysis of selected samples is gratefully acknowledged. The GC-MS analysis of other samples was performed by C. A. McDaniel of the U.S. Department of Agriculture, Gulfport, Mississippi.

SECTION 1

INTRODUCTION

In December 1974, an undetermined amount of wood-treating wastes containing pentachlorophenol (PCP) in fuel oil overflowed the banks of a wood-treatment company's waste water holding pond and soaked into soil and leaf litter throughout the spill area (Figure 1). The oil and PCP waste entered a small creek and traveled about one kilometer to a sixty-acre freshwater lake near Hattiesburg, Mississippi where the resulting fish kill was described as extensive to total (Mississippi Air and Water Pollution Control Commission, 1975). In December 1976 another extensive fish kill was observed in the lake and subsequent analyses showed that the fish died from acute PCP poisoning. The investigation reported here was undertaken to study the persistence and distribution of PCP and major PCP-degradation products and impurities in the aquatic ecosystem.

Pentachlorophenol (I) and the sodium salt, sodium pentachlorophenate (II), are pesticides that are used extensively for many industrial and agricultural applications. The most important uses include: as a fungicide and bactericide in processing textiles, paints, rubber and food; as a mulluscacide to control snails; as a herbicide; and, most extensively, as a wood preservative (Bevenue and Beckman, 1967). It is highly toxic to fish and other aquatic organisms with a median lethal concentration (LC-50) of 0.2 to 0.6 ppm (Coté, 1972; Cardwell et al., 1976) and accumulation also has been observed in livestock and in humans (Plimmer, 1973; Shafik, 1973; Dougherty and Pitrowska,

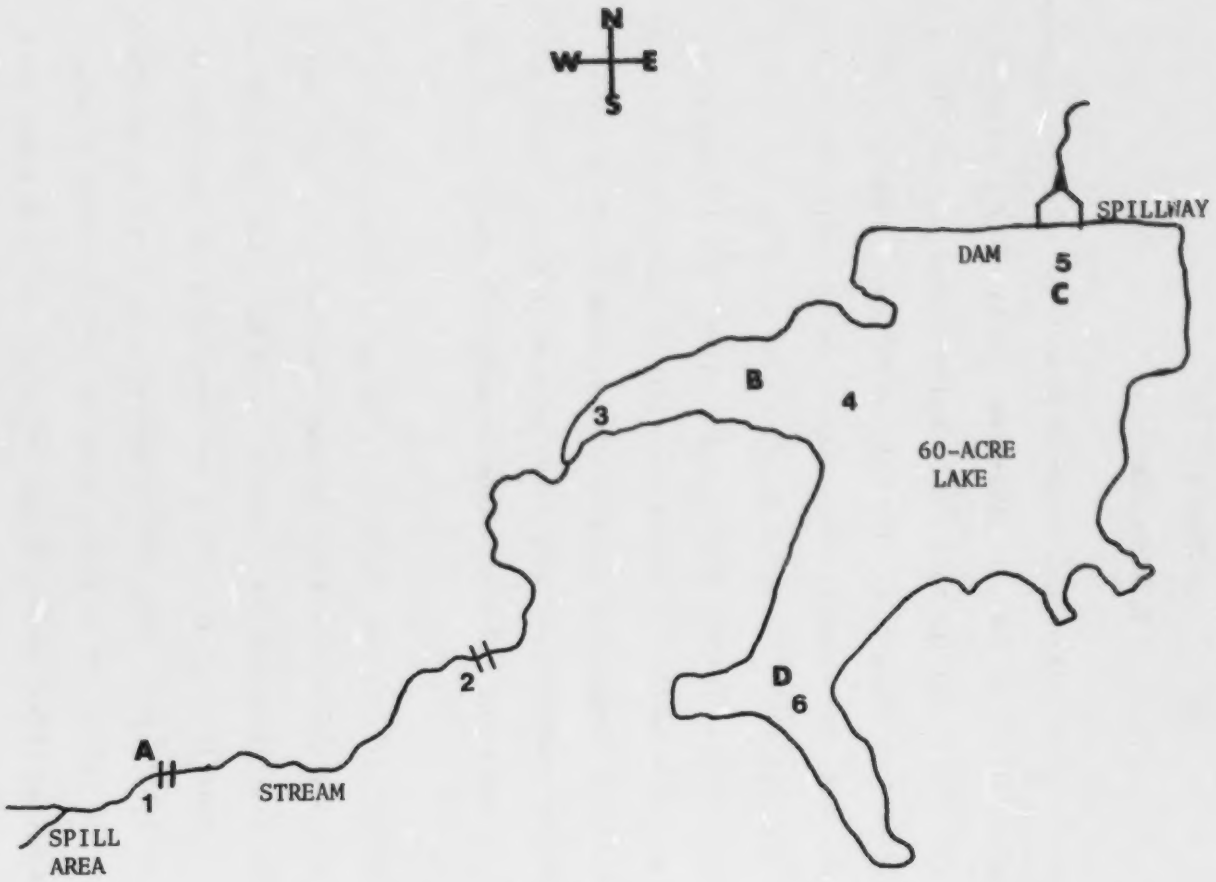
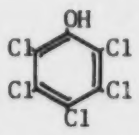
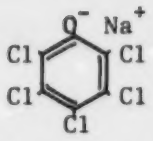


Figure 1. Sample sites in contaminated stream and lake: first year - 1,2,3,4,5,6; second year - A,B,C,D.

1976). Human illness and death have occurred from improper handling of PCP-treated lumber, from breathing PCP-contaminated sawdust, and from exposure when spraying solutions of PCP in fuel oil as a herbicide (Bevenue and Beckman, 1967; Shafik, 1973; Arsenault, 1976).



(I)



(II)

In addition to acute PCP poisoning, there is concern for contamination and biological magnification of PCP in aquatic organisms resulting from chronic exposure (Rudling, 1970; Stark, 1969; Buhler et al., 1973; Zitko et al., 1974; Kobayashi et al., 1976). Although the photo- and microbial-degradation of PCP has been observed to occur rapidly under controlled laboratory conditions (Crosby and Wong, 1976; Kirsch and Etzel, 1973), the pesticide has been found to persist in natural aquatic environments. The problem is magnified by the persistence of PCP-impurities and degradation products, many of which are also highly toxic, such as tetrachlorophenol (TCP), tetrachlorobenzo-ρ-quinone (chloranil), tetrachlororesorcinol, polychlorinated dibenzo-ρ-dioxins (PCDD) and polychlorinated dibenzofurans (PCDF) (Munakata and Kuwahara, 1969; Crosby et al., 1973; Crummett and Stehl, 1973; Rappe and Nilsson, 1972; Buser, 1976).

This two-year investigation has focused on the concentration of PCP and major PCP-impurities and degradation products in water, sediment, and fish in the contaminated ecosystem. In addition to investigating the fate of PCP, other studies have been performed to gain insight into the overall effect of PCP contamination. Food chain relationships were studied by observing benthic invertebrate populations in sediment and the stomach content of fish caught

in the lake. The accumulation and elimination of PCP by one of the major species of fish in the lake, the bluegill (Lepomis macrochirus), was observed under controlled laboratory conditions. Leaf litter, contaminated with PCP-in-oil, was washed in water to determine the rate at which PCP might be released from a contaminated water shed area. The solubility of PCP in water at various pH levels was also observed.

Routine collection of environmental samples was initiated in February 1975, two months after the PCP spill and fish kill. The project received funding in July 1975 and analysis of the environmental samples was initiated in November 1975.

SECTION 2

CONCLUSIONS

Pentachlorophenol concentrations in the water column and in fish remained high for several months following each fish kill; leaf litter from the contaminated water shed area and lake sediment contained high concentrations throughout the two-year study period.

The major degradation products observed were PCP-OCH_3, 2,3,5,6-TCP, and 2,3,4,5-TCP. The TCP isomers appeared to have been formed from PCP in the oil solution, prior to entering the lake, whereas PCP-OCH_3 seemed to have been formed within the aquatic ecosystem, and within leaf litter along the stream bank.

Fish rapidly accumulated PCP and PCP-degradation products from the contaminated lake with the largest concentrations observed in bile, followed by liver, gills, and muscle tissue. Laboratory experiments showed that the LC-50 for the bluegill, Lepomis macrochirus, was 0.3 ppm. Fish exposed to sublethal concentrations rapidly accumulated PCP, but were able to eliminate most of it within sixteen days when placed in a clean environment. Thus, the persistence of PCP in lake fish for several months following each spill indicates that the fish were subjected to chronic PCP contamination over an extended period of time.

At the pH observed for the lake water, PCP existed in the soluble phenate anion form, thus it was readily available for uptake by aquatic organisms A small amount was associated with particulate matter which may have provided an important means for incorporation into sediments.

Food chain studies showed that all gamefish depended upon benthic organisms either as a direct or indirect food source. Therefore, PCP and PCP-degradation product accumulation in sediments provided a continuous source for contamination of fish caught for human consumption. Questions remain regarding the persistence of PCP in sediment, since a continuous influx of PCP from the contaminated water shed could have given the appearance of PCP persistence in the sediment. In either case the sediment received continuous exposure to PCP and further study is needed to determine the long-term fate of PCP in the sedimentary environment.

In general, the results indicate that PCP released into the aquatic ecosystem was not rapidly assimilated by photo- or microbial-degradation. Acute toxicity to fish occurred by rapid uptake of the water soluble phenate anion, whereas chronic exposure occurred by leaching of PCP from the contaminated water shed into the lake and by incorporation of PCP into the benthic food chain. Thus, once contaminated with PCP, the sediment and water shed area provided a source for chronic PCP pollution of the aquatic ecosystem.

SECTION 3

RECOMMENDATIONS

Having ascertained the persistence of PCP in various parts of the ecosystem and observed major PCP-degradation products, it would now be advantageous to study individual aspects of the project more intensely both in the contaminated lake and under controlled laboratory conditions to gain a better understanding of the factors involved in the accumulation and degradation of PCP in the aquatic environments.

Of primary concern would be the partitioning of PCP between oil and water and the adsorption of PCP to suspended particulate matter. The photodegradation of PCP in fuel oil and volatilization of resulting products would be of concern to companies utilizing evaporation as their method of waste water disposal. The formation and persistence of minor degradation products which have been reported from previous laboratory experiments (i.e. tetrachlorohydroxyquinone, tetrachlororesorcinol, PCDD and PCDF) should be monitored in the contaminated ecosystem and their accumulation in fish should be studied to determine if they present a possible hazard to the ecosystem or to people using the lake.

Further study is needed on the accumulation and metabolism of PCP in fish including uptake via food as well as through the gills. Associated with this would be the accumulation of PCP in benthic organisms and their role in PCP bioaccumulation. The relatively high concentration of PCP-like compounds in the liver of supposedly non-contaminated fish should also receive more

attention. Additional study of PCP in fish should include the conjugated forms of PCP that are known to exist in liver and bile.

Questions remain regarding the composition and amount of the original spill and the frequency with which additional PCP-containing waste was released from the holding pond. The apparent persistence of PCP in lake sediment alternatively could have resulted from a continuous influx of PCP from the timber-treating company's operation. Frequent monitoring of the waste water holding pond and of runoff from the holding pond area would have been necessary to determine the extent of PCP influx to the lake. Further study is needed of the persistence of PCP in lake sediment, of degradation products, and of the potential for continuous contamination of the aquatic ecosystem via the benthic food chain, resuspension, and dissolution in the water.

SECTION 4

MATERIALS AND METHODS

ENVIRONMENTAL SAMPLES

Samples of the oil slick and a few random environmental samples were obtained immediately after the spill in December 1974. Routine sampling was initiated in February 1975 and samples were stored frozen until analysis began in November 1975, after the necessary equipment had been received and the reagent blanks and efficiency of the extraction and analysis procedures had been established. Extraction and analytical techniques were developed and improved throughout the first year. These procedural improvements along with information about the major PCP-degradation products helped to develop an improved study for the second year. Analytical procedures were modifications of those described by the U.S. Environmental Protection Agency (1974).

During the first year of the study, samples of water and sediment were obtained bi-monthly from six sites (Figure 1). Leaf litter was collected along the stream bank at site 1 near the spill area and fish were collected by seine along the lake shore near sites 3 and 5. Water quality parameters observed were temperature, hydrogen ion activity (pH), dissolved oxygen, turbidity, and total organic carbon.

Water samples were collected in 4-liter glass jugs with aluminum-lined caps. Dissolved and particulate PCP were separated by filtering the water through Reeve Angel grade 934-AH glass-fiber filter pads. Dissolved PCP was recovered by acidifying 2 liters of the filtrate to pH 2 with HCl and extract-

ing with 2 x 50 ml of benzene. Particulate PCP was recovered from the filter pads by washing with 0.1 N HCl, extracting the pads in benzene with ultrasonication for 15 minutes, followed by rinsing the pads with hexane. The benzene and hexane solutions were then combined. Sediment samples were collected with three grabs using an Eckman dredge to provide at least 1 kg of wet sediment from each site. The samples were stored frozen in aluminum-lined freezer containers, extracted by ultrasonication in benzene, and analyzed as described above for particulate PCP. Leaf litter was air-dried, blended to small pieces, and analyzed as described above for sediment.

Fish collected by seine from the two sites were combined, chopped into small pieces, and air-dried. A 25-g sample of air-dried, chopped fish was blended with 25 g Na_2SO_4, extracted into 100 ml benzene with ultrasonication, and filtered. The benzene extraction was repeated, the filtered residue washed with hexane, and the benzene and hexane solutions were combined.

The resulting benzene and hexane solution from each of the above sample extractions was washed with 0.1 N NaOH to separate phenolic compounds from base-insoluble components. The aqueous solution was then acidified to pH 2 with HCl and the phenols were extracted into hexane. The PCP content of the resulting hexane solution was determined by gas chromatographic analysis utilizing electron capture (EC-GC) detectors. Samples were analyzed before and after methylation with diazomethane according to the procedure of Schlenk and Gellerman (1960).

During the second year of the study (July 1976 to July 1977), improved sample collection and analysis techniques were utilized allowing more involved analyses to be performed on the samples. Second-year samples were collected from four sites on a quarterly basis (Figure 1). Fish were collected near site B by gill net to obtain large specimens so that various organs of the fish

could be analyzed separately. Fish tissue was analyzed by lysing 1 g (wet weight) in 2 ml of 50% H_2SO_4, adding 2 ml acetonitrile, and extracting with 2 x 5 ml hexane. The hexane solution (hexane-I) was then washed with distilled water and the base-soluble components (phenols) were extracted with 2 x 5 ml of 0.1 N NaOH, the aqueous HaOH solution was then acidified to pH 2 with HCl and the phenols were extracted with 2 x 5 ml hexane (hexane II). Both hexane-I (containing base-insoluble components) and hexane-II (containing base-soluble components) were then analyzed by GC-EC before and after methylation with diazomethane. The basic extraction procedure used during the second year of the study is shown in Figure 2.

Water samples were analyzed in triplicate by acidifying filtered 1-liter samples to pH 2 and extracting with 2 x 25 ml hexane. The hexane extract was separated into hexane-I and hexane-II and analyzed as described above for fish samples. Sediment samples were air-dried, washed with 0.1 N HCl and extracted with a solution of acetone/hexane (60/40, v/v) under reflux for 20 hours. The acetone was removed by washing with water and the hexane solution recovered and treated as described above. Particulate matter, collected on filter pads, and leaf litter were analyzed by the same method as sediments.

The base-insoluble components (hexane-I solution) from selected samples were further fractionated by elution through an alumina micro-column (Buser, 1975) in an attempt to isolate and identify dioxins and dibenzofurans. Control samples of water, sediment, leaf litter, and fish were concurrently collected from an isolated 5-acre pond which received no industrial or agricultural drainage. Levels of PCP and PCP-degradation products in the control pond are considered to represent background concentrations in this area. Efficiency

of the extraction procedures was determined by the addition of known amounts of PCP to clean samples and subjecting the "spiked" samples to the extraction and analysis procedures.

Samples were analyzed with a Varian model 2700 gas chromatograph with $Sc^{3}H$ electron capture detectors. Two 3 mm x 2 m stainless steel columns were used for identification: a non-polar 3% SP-2100 on 80/100 Supelcoport and a polar 10% SP-1000 on 80/1000 chromosorb W.A.W. Injector temperature was

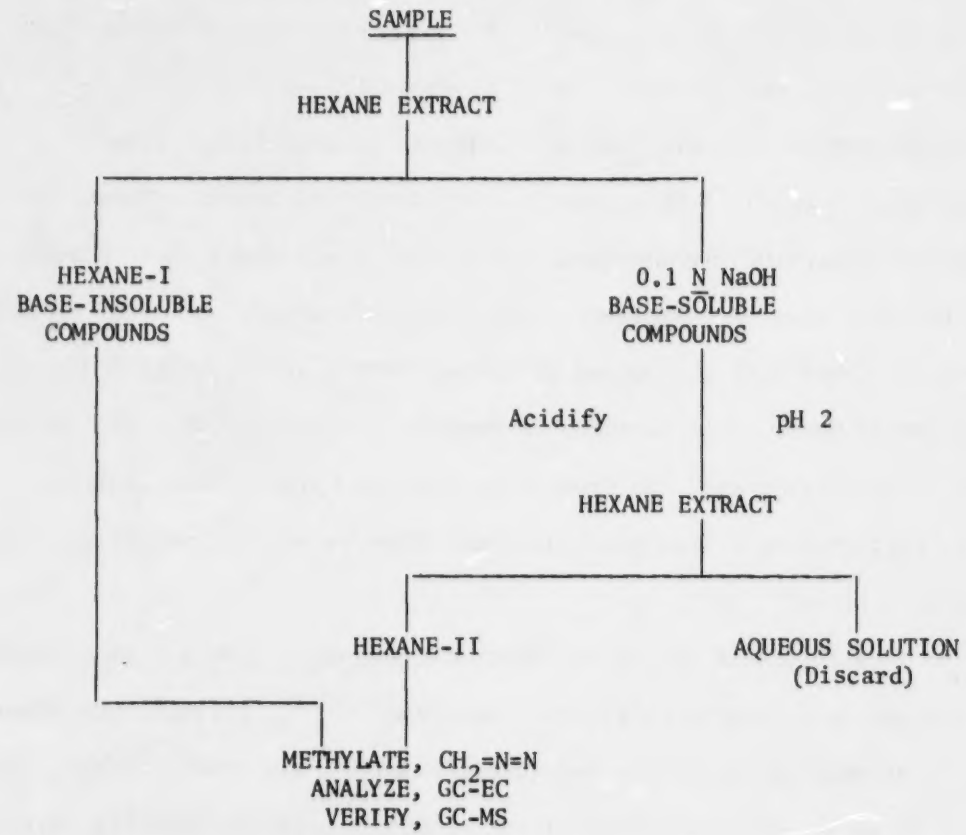


Figure 2. Extraction of PCP and PCP-degradation products from environmental samples.

175°C, column 200°C, and detector 250°C. The carrier was N_2 at a flow rate of 25 ml/min. Compound identity was verified from representative samples utilizing a Hewlett-Packard 5933 gas chromatography-mass spectrometry (GC-MS) data system. All solvents were reagent grade, redistilled in a glass distillation apparatus and tested for purity by concentrating 100 fold, methylating and analyzing by GC-EC.

PCP RELEASE FROM CONTAMINATED LEAF LITTER

Leaf litter collected from the stream bank at site 1 on February 27, 1975, was used to study the release of PCP from contaminated leaf litter into water. Three replicate experiments were performed in which 1 g of air-dried leaf litter was placed in 100 ml of distilled water and shaken at 60 rpm for 24 hours. The water was then decanted through medium-porosity glass fiber filter pads and saved for PCP analysis.

The filter pad was then back-washed with 100 ml of distilled water and the water added to the leaf litter for an additional 24-hour period of shaking. The process was repeated and after the third 24-hour equilibrium period, the three separate water samples and the leaf litter were analyzed for PCP content according to the procedures described above for the first year study of environmental samples.

SOLUBILITY OF PCP IN WATER

Solubility in Distilled Water

The solubility of PCP in distilled water was determined by adding excess reagent grade PCP to 250 ml distilled water and enhancing dissolution by ultrasonication or stirring up to 62 hours. The concentration of the molecular (phenol) and ionic (phenate) forms of PCP in aqueous solution was determined

by titrating a solution of PCP with HCl and obtaining the spectrophotometric curve of the solution from 400 to 200 nm as increments of HCl were added. To calculate the concentration of the above forms at equilibrium, the absorbance maxima at 219 nm was observed after the addition of 0.09 M HCl. Assuming that the concentration of the phenate anion is represented by the absorbance maximum ($A_{319\text{-max}}$) at pH 12, then the quantity of phenol may be represented by ($A_{319\text{-max}} - A_{319}$) where A_{319} is the absorbance at a lower pH. The dissociation constant is then calculated according to equation (1).

$$\frac{Ka}{[H^+]} = \frac{A_{319}}{(A_{319\text{-max}} - A_{319})} \qquad (1)$$

The pH was monitored with a Corning Model 12 pH meter with glass vs. calomel electrodes, and spectral data was obtained with a Cary 17 spectrophotometer utilizing 1-cm and 10-cm cells.

Solubility in Buffered Solutions

Since natural aquatic systems usually have some buffer capacity (i.e. alkalinity), the study was expanded to determine the solubility of PCP in buffered solutions at pH 2.2, 6.6, and 10.1. The pH 2.2 buffer solution was prepared by mixing 0.1 M H_3PO_4 and 0.1 M KH_2PO_4 until the desired pH was obtained. Buffer solutions at pH 6.6 were prepared from 0.1 M KH_2PO_4 and 0.1 M NaOH, whereas pH 10.1 buffers were made from 0.1 M NaOH and 0.1 M H_3BO_3.

To determine absorption maxima in the ultra-violet (u.v.), buffered samples were prepared in pH 2.2 buffer for PCP and in pH 10.1 buffer for sodium pentachlorophenate (SPCP). Spectrophotometric curves from 200 to 360 nm were determined using a Bausch & Lomb Spectronic 710.

Two methods were used to prepare PCP solutions for solubility studies. In the first, a quantity of PCP was weighed out; to this was added a known volume

of prepared buffer. Each flask, including blanks and standard recoveries, was then closed with an aluminum-foil covered stopper; wrapped with foil to exclude light; and shaken for 18 hours or more at 120 rpm and 25-28°C on a New Brunswick Scienctific Controlled Environment Incubator (New Brunswick, N.J.).

In the second method, 0.1, N NaOH was added to dissolve a known quantity of PCP. Acid was then added to the SPCP solution to bring the pH to the desired level. Flasks were then covered with aluminum foil and treated as in the first method.

Flasks were removed from the shaker, and aliquots transferred to glass centrifuge tubes to be spun down in either a Model CL International Clinical Centrifuge for 10 minutes or a Sorvall General Laboratory Centrifuge at 4700 rpm for 10 minutes. Unwetted, floating PCP was removed from the solution surface and sample aliquots were then taken from beneath the surface.

Sample aliquots of 50 ml each were extracted using 125-ml separatory funnels, and 1 ml aliquots were extracted in 15 x 150 mm test tubes using a vortex mixer. Sulfuric acid was added to each sample, where necessary, to bring the pH to 2. Samples were then extracted with two aliquots of hexane, and the aqueous phase was discarded. The combined hexane portions were then washed with distilled water, and the water was discarded. Then the hexane phase was washed with two aliquots of 0.1 N NaOH and the hexane was set aside as hexane-I. The combined basic solutions were then acidified to pH 2 with sulfuric acid and extracted with two aliquots of hexane. These two aliquots were combined and set aside as hexane-II.

For gas chromatographic analysis, samples containing phenols were concentrated to about 5 ml under a stream of N_2 and then subjected to methylation according to the method of Schlenk and Gellerman (1960). Samples were

allowed about 5 minutes to react and then were brought to an appropriate volume for gas chromatographic analysis as described above for environmental samples.

ACCUMULATION AND ELIMINATION OF PCP IN FISH

Six-month-old bluegill, Lepomis machrochirus, were obtained from the Lyman Fish Hatchery, Lyman, Mississippi. The fish were acclimated to laboratory conditions for a two-week period, randomly divided into test groups of ten to thirty in 30-liter glass aquaria and re-acclimated for 24 hours with no feeding before initiating toxicity and exposure studies.

The studies were performed as static bioassays in dechlorinated tap water. Each aquaria was monitored at the beginning and end of each 24-hour period for pH, temperature, conductivity, and dissolved oxygen. The PCP content was measured in each aquarium after the initial 24-hour period to verify the desired exposure level. The test solutions were changed every 24 hours thereafter to compensate for PCP loss by evaporation, adsorption, and decomposition. Control aquaria were treated in the same manner, without PCP.

Standard stock solutions of PCP were prepared by two procedures. A PCP standard was made by dissolving 1 g reagent grade PCP in 1 liter of acetone. An SPCP standard (for sodium pentachlorophenate) was prepared by dissolving 1 g PCP in an aqueous NaOH solution at pH 12 and bringing the pH to 7.4 with the addition of 0.1 M H_3PO_4. All exposure studies were performed with the PCP standard solution.

Before initiating exposure studies, the toxicity of PCP for L. machrochirus was verified using 96-hour median lethal concentration (LC-50) static bioassays according to the procedure in Standard Methods (American Public Health Association, 1971). Ten fish were placed in each test aquaria and ten for control. Percent survival was observed at the end of each 24-hour period.

Accumulation studies were performed in three replicate aquaria containing thirty fish each along with a control aquaria. The fish were exposed to a sublethal concentration of 0.1 ppm PCP and two fish from each aquarium were removed for analysis on day 1, 2, 4, 8, and 16 of the exposure period. A 1-g sample of muscle tissue and the combined gills, liver, and digestive tract were analyzed for PCP content for each fish. On days 4, 8, and 16, three additional fish were killed for separate analysis of gills, liver, and digestive tract.

Elimination studies were initiated by placing fish exposed to 0.1 ppm PCP in clean water, which was changed every 24 hours. These studies were performed in triplicate aquaria versus control for each of two groups of fish. Group-I fish (short-term exposure) were placed in clean water after 4 days exposure and group-II fish (long-term exposure) were placed in clean water after 16 days exposure.

Group-I fish were collected on day 1, 2, 4, 8, and 16 and the muscle and combined gills, liver, and digestive tract were analyzed. Group-II fish were collected on day 2, 4, 8, and 16 for muscle and combined tissue analysis of the muscle, gills, liver, and digestive tract.

Fish Collected for PCP analysis were weighed and measured prior to dissection. Tissues to be analyzed were weighed and placed in 30-ml vials. The PCP content was determined in a manner similar to that described above for fish tissue analysis of environmental samples.

FOOD CHAIN STUDY

Benthic collections were taken quarterly at four stations during the second year of the project. Collections consisted of three (15.5 cm x 15.5 cm) samples from an Eckman dredge at each station. Samples were placed in plastic

bags and packed in ice. After returning to the laboratory, samples were fixed in a ten percent formalin solution. Organisms were separated from the substrate by passing samples through a wire sieve (U.S. Standard No. 50). Benthic invertebrates were then separated from the strained material, identified and volume determined.

Fish analyzed in food habit studies were captured by both trawl and gill net. Immediately after capture fish were placed on ice and returned to the laboratory. Stomachs were removed and the species and length of fish recorded. Stomachs were then placed in ten percent formalin until examination. Food items were identified in the same manner as the benthic samples. Analysis of the stomach contents includes the determination of the number, volume and frequency of occurrence of each food item.

SECTION 5

RESULTS AND DISCUSSION

ENVIRONMENTAL SAMPLES

Low levels of PCP and PCP-degradation products were observed in the control pond samples (TABLE 1) exhibiting concentrations of 0.3 ppb in water, 3 ppb in sediment, 7 ppb in fish muscle, and 70 ppb in fish liver. Reagent blanks were well below the PCP concentration observed in control samples indicating that a slight background concentration of PCP did exist in the supposedly non-contaminated environment samples. This background contamination could be due to atmospheric transport of PCP from treatment sites. It has also been suggested (Arsenault, 1976), that a naturally occurring fungal metabolite, p-methoxytetrachlorophenol could be mistaken for low levels of PCP in some samples.

Procedures utilized during the first year study provided the following percent recoveries ± one standard deviation: water, 90 ± 15; sediment, 90 ± 40; fish, 62 ± 14. Second-year procedures provided the following extraction efficiencies: water, 95 ± 10; sediment 110 ± 25; fish, 90 ± 8.

PCP Persistence and Distribution

The investigation during the first year of sampling, concerned primarily with the distribution and persistence of PCP in the lake, has been discussed by Pierce et al. (1977) and will be summarized here in relation to the rest of the study. Within two months after the spill, PCP was uniformly distributed throughout the lake at a concentration of 10 ppb in February 1975

TABLE 1. PCP AND PCP-DEGRADATION PRODUCTS IN CONTROL SAMPLES.[a]

Sample	PCP	PCP-OCH_3	2,3,5,6-TCP	2,3,4,5-TCP
Water[b]				
Dissolved	0.3±0.1	<0.01[c]	0.1±0.01	<0.01
Particulate	0.1±0.05	<0.1	<0.1	<0.1
Sediment[d]	3.0±0.4	<0.1	1.0±0.2	<0.1
Fish[e]				
Muscle	7.0±3	<1	<1	<1
Liver	70.0±40	<1	<1	<1
Leaf Litter[f]	5.0±1	5.0±2	7.0±2	3.0±1

[a]Average of duplicate analysis ± ½ the range.

[b]Concentrations reported as µg/liter.

[c]Lower limit of detection.

[d]Concentrations reported as µg/kg air-dry sediment.

[e]Concentrations reported as µg/kg wet-weight tissue.

[f]Concentrations reported as µg/kg air-dry leaf litter.

(TABLE 2). The concentration decreased to background levels (<1 ppb) by October 1975, increased again to about 15 ppb in February 1976, and steadily declined through May 1976. Pentachlorophenol associated with particulate matter usually represented less than ten percent of the dissolved PCP (TABLE 2). Water samples collected during the second year (TABLE 3) showed background

TABLE 2. PCP CONCENTRATION IN WATER SAMPLES COLLECTED FEBRUARY 1975 THROUGH MAY 1976.

Date Sample	Site 1	2	3	4	5	6
			µg PCP/liter			
Feb. 27, 1975						
Dissolved	9	11	6	9	9	8
Particulate	0.2	0.4	0.3	0.4	0.6	0.2
Apr. 24, 1975						
Dissolved	11	6	8	8	9	15
Particulate	3	N.A.[a]	0.2	0.1	0.2	N.A.
June 28, 1975						
Dissolved	13	8	2	3	2	82
Particulate	2	0.2	1	2	0.2	0.4
Aug. 5, 1975						
Dissolved	N.A.	N.A.	3	2	3	1
Particulate	N.A.	N.A.	0.3	0.4	0.2	N.A.
Oct. 11, 1975						
Dissolved	10	5	0.8	0.1	1.0	0.2
Particulate	2	0.3	<0.1[b]	<0.1	0.1	<0.1
Dec. 6, 1975						
Dissolved	76	25	1	2	2	N.A.
Particulate	5	0.4	<0.1	<0.1	<0.1	N.A.
Feb. 7, 1976						
Dissolved	29	N.A.	15	10	10	26
May 3, 1976						
Dissolved	18	N.A.	2	N.A.	2	2

[a] N.A. = Not Analyzed.
[b] Lower limit of detection.

levels in lake water in August and October 1976, high concentrations in January and February 1977, and decreasing somewhat, yet still well above background in April 1977. Although suspended particulate matter contained less than ten percent of the PCP in the water column throughout the study, it may be important as a transport mechanism from the water column to sediment.

TABLE 3. PCP IN WATER SAMPLES COLLECTED AUGUST 1976 THROUGH APRIL 1977.

Date	Sample	Site A	B	C	D
		µg PCP/liter			
Aug. 11, 1976					
	Dissolved[a]	11.0±0.4	0.1±0.02	0.1±0.03	0.1±0.01
	Particulate[b]	0.2	0.06	0.05	0.06
Oct. 22, 1976					
	Dissolved	N.A.[c]	0.1±0.03	0.2±0.05	1.0±0.1
	Particulate	N.A.	0.04	0.04	0.2
Jan. 5, 1977					
	Dissolved	82.0±32	24.0±13	25.0±7	16.0±6
	Particulate	0.4	N.A.	0.2	0.2
Feb. 22, 1977					
	Dissolved	146.0 7	N.A.	29.0±2	N.A.
	Particulate	0.18	N.A.	0.14	N.A.
Apr. 27, 1977					
	Dissolved	16.0±0.4	5.0±0.5	5.0±0.7	5.0±0.2
	Particulate	0.33	0.29	0.35	0.29

[a]Mean of triplicate analyses ± sandard deviation.

[b]Composite of all filter pads from three one-liter samples.

[c]N.A. = Not Analyzed.

Water quality parameters monitored for February 1975, through May 1976, (TABLE A-1) show that the pH remained slightly acidic in the lake, generally between pH 6.0 to 6.8 but was more acidic in the shallow stream (4.2 to 6.2). Near-surface water was well oxygenated, except in the shallow stream when the water was stationary and varied seasonally with temperature. Total organic carbon (TOC) remained fairly constant throughout the study period except for anomolously low concentrations observed in April 1975.

During the second year of the study, dissolved oxygen (D.O.) and temperature were monitored near the surface and near the bottom at each site, but pH was recorded for surface samples only (TABLE A-2). Surface samples exhibited conditions similar to the previous year, but the bottom samples revealed anoxic conditions existing at the bottom of site C for the summer, fall, and spring sample periods.

Sediment samples retained PCP concentrations well above background throughout the first year of the study (TABLE 4). The variability observed among these samples may be attributed to the inefficiency of the sonication extraction procedure, compounded by variation in the environmental samples. The second-year sediment samples (TABLE 5) show that large concentrations of PCP remained in sediments throughout the study period.

Sediment contained high PCP concentrations in August and October 1976 (500 ppb average) and exhibited a slight decrease in January 1977 (200 ppb), after the second fish kill, indicating a residence time of over a week in the water column before incorporation into sediment. Lake sediment showed an increase near the mouth of the stream in February 1977 (1,500 ppb) but in April 1977 the concentration returned to the January levels (250 ppb).

TABLE 4. PCP CONCENTRATION IN SEDIMENT SAMPLES COLLECTED FEBRUARY 1975 THROUGH MAY 1976.[a]

Date	Site 1	2	3	4	5	6
	µg PCP/kg air-dry sediment					
Feb. 27, 1975	800	1[b]	22	26	119	2
Apr. 24, 1975	1,160	36	21	98	180	34
June 28, 1975	1,300	1	1.4	583	860	92
Aug. 5, 1975	N.A.[c]	N.A.	10	205	207	45
Oct. 11, 1975	927	471	48	91	56	24
Dec. 6, 1975	163	900	11	97	97	N.A.
Feb. 7, 1976	100	N.A.	313	10	84	81
May 3, 1976	96	N.A.	20	N.A.	21	7

[a]Composite of three grab samples.

[b]Lower limit of detection.

[c]Not analyzed.

Small fish collected by seine during the first year showed high whole-body PCP concentrations in February (2,500 ppb dry-weight) and April 1975 (TABLE 6). The concentration was diminished yet still above background in June 1975, increased some in December 1975 and February 1976, and then decreased again to background by May 1976 (TABLE 6). Improved sampling and analytical procedures used for the second year of the study provided a more complete description of the accumulation of PCP in fish. These results (TABLE 7) show that fish contained only background levels of PCP in October 1976 but rapidly accumulated very high concentrations in January 1977 immediately after the

TABLE 5. PCP IN SEDIMENT SAMPLES COLLECTED AUGUST 1976 THROUGH APRIL 1977.

Date	Site A	B	C	D
	ug/kg air-dry sediment[a]			
Aug. 11, 1976	857±57	429±169	520±151	142±44
Oct. 22, 1976	166±38	994±394	389±12	212±8
Jan. 5, 1977	277±53	239±75	150±13	170±78
Feb. 22, 1977	N.A.[b]	1,518±87	N.A.	N.A.
Apr. 27, 1977	4±1	250±14	238±3	132±21

[a]Average of duplicate analyses ± ½ range.

[b]Not analyzed.

TABLE 6. PCP IN FISH COLLECTED FEBRUARY 1975 THROUGH MAY 1976.

Collection Date	ng PCP/g air-dry fish tissue[a]
Feb. 27, 1975	2,500±200
Apr. 24, 1975	1,380±20
June 23, 1975	130±70
Oct. 11, 1975	<50[b]
Dec. 6, 1975	651±650
Feb. 2, 1976	87±22
May 3, 1976	<50

[a]Average of replicate analyses ± ½ the range.

[b]Lower limit of detection.

TABLE 7. PCP IN FISH COLLECTED
AUGUST 1976 THROUGH APRIL 1977.

Fish		Length cm	Tissue: Muscle	Gills	Liver	Bile/(Stomach)
			ng PCP/g wet weight tissue[a]			
			August 11, 1976			
Sunfish	(3)[b]	5-10	51±15	N.A.[c]	600	(77)
			October 22, 1976			
Sunfish	(2)	12-15	5±1	N.A.	90±60	N.A.
Catfish	(2)	25-26	5±1	N.A.	87±50	N.A.
Shiner[b]	(1)	10	55	N.A.	N.A.	(304)
Sucker[b]	(1)	20	14	N.A.	380	(330)
			January 6, 1977			
Sunfish	(2)	15-20	8,000±1,000	48,400	100,000	N.A.
Bass	(3)	36-44	13,000±3,000	42,000±100	230,000±70,000	2,100,000±400,000
Catfish[b]	(1)	25	4,000	N.A.	213,000	N.A.
Shiner	(2)	10-12	4,800±400	N.A.	N.A.	(140,000±30,000)
Sucker[b]	(1)	22	7,000	35,000	466,000	N.A.
			April 27, 1977			
Sunfish	(2)	14-20	980±90	N.A.	14,750±150	N.A.
Catfish	(2)	40	5,000±3,000	N.A.	35,000±15,000	150,000±50,000

[a]Average of replicate analyses ± ½ the range.

[b]Number of fish analyzed.

[c]Not analyzed.

spill. Concentrations decreased somewhat by April 1977 but were still well above background levels. Bass collected freshly dead or dying in January 1977 shortly after the spill, contained PCP in concentration of 2,100,000 ppb in bile; 230,000 ppb in liver tissue; 42,000 ppb in gills; and 13,000 ppb in muscle. These values represent concentration factors over the PCP content in water of 500 for muscle; 1,500 for gills; 8,000 for liver; and 80,000 for bile.

Leaf litter collected near the spill site contained very high concentrations of PCP throughout the investigation (TABLE 8). The variability observed between sampling dates probably reflects the heterogeneous nature of the collection area, but the large concentrations observed overall indicate that contaminated

TABLE 8. PCP IN LEAF LITTER.

Date	PCP ng/g air-dry leaves[a]
Feb. 27, 1975	6,400±250
Apr. 24, 1975	2,550±650
June 23, 1975	5,200±300
Aug. 11, 1975	5,800±1,200
Dec. 6, 1975	3,470
Feb. 10, 1976	1,680±12
May 3, 1976	6,000±1,000
Aug. 11, 1976	10,300±1,000
Oct. 22, 1976	3,149±380
Jan. 5, 1977	15,900±180
Apr. 27, 1977	2,970±800

[a]Average of duplicate analyses ± ½ the range.

leaf litter provided a potential for long-term pollution of the aquatic ecosystem.

PCP Degradation Products

Since the source of PCP contamination was the oil and water from the industrial waste water holding pond, samples of oil and water from the pond and oil from the surface of the stream near the spill site were collected and analyzed for PCP and PCP-degradation products. The results (TABLE 9) show that the PCP concentration was 617,000 ppb in the oil slick from the stream; 220,000 ppb in holding pond oil; and 8,700 ppb in holding pond water. The major degradation products observed in the oil slick were the 2,3,4,5- and 2,3,5,6-TCP isomers, each approximately 13% of the PCP concentrate (TABLE 9). These percentages far exceeded the percent TCP observed in a sample of technical grade PCP (1 to 2%) suggesting that both isomers of TCP were formed from PCP in the oil solution, probably by photodegradation as suggested by Crosby (1972).

The major degradation products observed in the contaminated lake were pentachloroanisole (PCP-OCH_3) and the two isomers of TCP. Varying quantities of the methyl ether (anisole) of both TCP isomers were also observed but proved difficult to quantitate, due to low concentrations and interference from naturally occurring substances. The 2,3,4,6-TCP isomer was not observed but may have been present in small quantities. Trichlorophenol eluted with the solvent front under the chromatographic conditions used, thus its concentration was not determined. The presence of chloranil, PCDD, and PCDF were indicated in sediment and holding pond oil samples in ug/kg quantities, utilizing the procedure described by Buser (1975), but our analytical system did not provide adequate quantitation. The gas chromatographic retention of the methylated TCP isomers relative to methylated PCP was observed to be 0.5 for 2,3,5,6-TCP

TABLE 9. PCP AND DEGRADATION PRODUCTS IN TECHNICAL GRADE PCP, INDUSTRY WASTE-HOLDING POND AND OIL SLICK FROM STREAM.

	PCP[a]	2,3,5,6-TCP[b]	2,3,4,5-TCP[b]
Technical-Grade PCP Solution	1,000	1.2%	<0.01%
Holding Pond[d]			
Oil Slick	220,000	13%	N.A.[c]
Water	8,700	9%	N.A.
Stream Oil Slick[e]	617,000	12%	13%

[a]ng PCP/ml solution (ppb).

[b]Reported as percentage relative to PCP concentration.

[c]Not analyzed.

[d]Collected from industrial waste holding pond, September 8, 1975.

[e]Colelcted from spill area, December 18, 1974.

and 0.80 for 2,3,4,5-TCP on 3% SP-2100, and 0.46 for 2,3,5,6-TCP and 1.21 for 2,3,4,5-TCP on 10% SP-1000. The relative response factor for peak height was 1.0 for 2,3,5,6-TCP and 0.25 for 2,3,4,5-TCP on 3% SP-2100; and 1.5 for 2,3,5,6-TCP and 0.25 for 2,3,4,5-TCP on 10% SP-1000.

Degradation products in control pond samples are given in TABLE 1. The concentrations were found to be essentially at reagent blank lavels in all samples except leaf litter, which exhibited background levels of all products in the 3-7 ug/kg range.

The concentration of degradation products dissolved in the water column are shown in TABLE 10. The PCP-OCH_3 remained at background levels throughout, reflecting the low solubility in water. The 2,3,5,6-TCP was below background in August and October, but increased to almost 1 ppb in January 1977 and remained above background through April 1977. The 2,3,4,5-TCP isomer remained below or near background throughout the sampling period.

Sediment retained high concentrations of all the degradation products throughout the August 1976 through April 1977 study period (TABLE 11). The TCP isomers were both more concentrated than PCP-OCH_3 from August through February, but the latter increased in April. This observation, along with the absence of PCP-OCH_3 in technical PCP or the oil slick suggests that PCP-OCH_3 was formed in the aquatic environment, probably by microbial action on PCP as reported by Cserjesi and Johnson (1972). The changes in TCP concentration followed a pattern similar to that for PCP, suggesting that TCP was formed before it reached the sedimentary environment.

TABLE 10. PCP-DEGRADATION PRODUCTS IN LAKE WATER (DISSOLVED) SAMPLES COLLECTED AUGUST 1976 THROUGH APRIL 1977.

Date	PCP-OCH_3	2,3,5,6-TCP ug PCP/liter[a]	2,3,4,5-TCP
Aug. 11, 1976	0.06±0.01	0.08±0.02	0.05±0.04
Oct. 22, 1976	0.04±0.01	0.06±0.02	0.03±0.01
Jan. 5, 1977	0.08±0.02	0.9±0.1	0.07±0.02
Feb. 22, 1977	0.07±0.03	0.3±0.02	0.05±0.02
Apr. 27, 1977	0.03±0.01	0.9±0.1	0.3±0.1

[a]Average of sites B, C, and D ± standard deviation.

TABLE 11. PCP-DEGRADATION PRODUCTS IN LAKE SEDIMENT COLLECTED AUGUST 1976 THROUGH APRIL 1977.

Date	PCP-OCH_3[a]	2,3,5,6-TCP[a]	2,3,4,5-TCP[b]
	ug PCP/kg air-dried sediment		
Aug. 11, 1976	18.0±5	120±60	129
Oct. 22, 1976	6.0±6	62±5	63
Jan. 5, 1977	16.0±1	50±20	24
Feb. 22, 1977	1.5±0.2	340±15	N.A.[c]
Apr. 27, 1977	60.0±20	53±25	15±3

[a]Average of values for sites B, C, and D ± standard deviation.

[b]Site B only.

[c]N.A. = not analyzed.

The concentrations of PCP-degradation products in various fish tissues are shown in TABLE 12 for samples collected in August and October 1976, and in TABLE 13 for fish collected in January and April 1977. Fish collected in August and October contained low concentrations. Samples collected in January 1977, shortly after the second fish kill, showed very high concentrations indicating that fish rapidly accumulated all products from the environment. The TCP isomers were found in increasing concentration in muscle, gills, liver, and bile, in the same manner as was observed for PCP. The PCP-OCH_3 was generally less concentrated than TCP and showed a marked reduction in bile, probably as a result of the insolubility in aqueous solution. The high concentration of PCP-OCH_3 in fish obtained from water containing very low concentrations indicates a very high partition coefficient for PCP-OCH_3 from water to fish. It is also possible that the fish received some of the PCP-OCH_3 in their diet.

The persistence of PCP and PCP-degradation products observed in fish indicate that the fish were exposed to high levels over an extended period of time and that the toxic chemicals were not rapidly eliminated but were retained, perhaps in a conjugated form, as reported by Kobayashi, et al. (1976).

TABLE 12. PCP-DEGRADATION PRODUCTS IN FISH COLLECTED AUGUST AND OCTOBER 1976.

Fish	(No.)	Size cm	$PCP\text{-}OCH_3$	2,3,5,6-TCP	2,3,4,5-TCP
			ng PCP/g wet-weight tissue[a]		
			August 11, 1976		
Sunfish	(2)	5-10			
		muscle	41±20	5±2	<1[c]
		liver[b]	600	92	130
			October 22, 1976		
Sunfish	(2)	12-15			
		muscle	3±1	<1	<1
		liver	11±1	40±10	5±1
Catfish	(2)	25-26			
		muscle	<1	1±0.6	<1
		liver	17±5	50±15	150±100
Sucker	(1)	20			
		muscle[b]	N.A.[d]	<1	<1
		liver[b]	30	260	285

[a]Average of replicate samples ± ½ the range.

[b]Analysis of single, composite sample.

[c]Lower limit of detection.

[d]Not analyzed.

TABLE 13. PCP-DEGRADATION PRODUCTS IN FISH COLLECTED JANUARY AND APRIL 1977.

Fish	(No.)	Size cm	$PCP\text{-}OCH_3$	2,3,5,6-TCP	2,3,4,5-TCP
			ng PCP/g wet-weight tissue[a]		
			January 6, 1977		
Sunfish[e]	(2)	15-20			
		muscle	60±30	75±15	<10[c]
		gills[b]	230	360	150
		liver[b]	560	950	300
Bass[e]	(3)	36-44			
		muscle	170 80	230 96	<10
		gills	60±8	335±35	N.A.[d]
		liver	600±200	5,600±2,000	500±200
		bile	208±8	114,000±10,000	6,000±500
Catfish[e]	(1)	25			
		muscle[b]	164	219	N.A.
		liver[b]	1,200	8,500	N.A.
Sucker[f]	(1)	22			
		muscle[b]	84	45	<10
		gills[b]	90	450	180
		liver[b]	490	11,360	3,200
			April 27, 1977		
Sunfish[f]	(2)	14-20			
		muscle	29±1	20±2	<10
		liver	155±35	200±50	30±10
Catfish[f]	(2)	40			
		muscle	140±35	60±20	<10
		liver	350±200	720±60	365±35
		bile	210±20	1,000±500	1,100±600

[a] Average of replicate samples ± ½ the range.

[b] Analysis of single or composite sample.

[c] Lower limit of detection.

[d] Not analyzed.

[e] Collected freshly dead or dying.

[f] Collected alive by gill net.

Since leaf litter contained very high concentrations of PCP, some of the minor degradation products (i.e., TCP-OCH_3) were observed which were not distinguishable in other environmental samples. The large concentration of the anisoles of PCP and both TCP isomers (TABLE 14) throughout the study period indicates that the process by which anisoles are formed occurred within the dried grass and leaf litter along the banks of the stream. The persistence of all products throughout the study period showed that the leaf litter was a source for continuous environmental pollution.

PCP RELEASE FROM CONTAMINATED LEAF LITTER

This experiment was devised to determine the extent to which contaminated leaf litter might release PCP to the water, thus providing a source for continuous pollution of the aquatic ecosystem. The results (TABLE 15) show that approximately ten percent of the PCP in the leaf litter was leached out over each 24-hour peiod of shaking in water. The original concentration of PCP in the leaf litter was 6.4 µg/g and that in the water after equilibrium was about 0.5 µg/g. Since the reported solubility of PCP in water is 15 ppm (Bevenue and Beckman, 1967), solubility would not have been a limiting factor. A mass balance (TABLE 15) shows that all of the PCP was accounted for in the experiment.

These data indicate that PCP associated with leaf litter and vegetation along the bank of a stream would be released slowly over a period of time. Therefore, contaminated leaf litter serves as a source for chronic PCP contamination of the aquatic environment. This finding is supported by the persistence of PCP in leaf litter throughout the study.

TABLE 14. PCP DEGRADATION PRODUCTS IN LEAF LITTER COLLECTED AUGUST 1976 THROUGH APRIL 1977.

Date	PCP-OCH_3	2,3,5,6-TCP	2,3,5,6-TCP-OCH_3	2,3,4,5-TCP	2,3,4,5-TCP-OCH_3
	ng PCP/g air-dried leaf litter[a]				
Aug. 11, 1976	280±48	448±100	74±5	332±16	52±40
Oct 22, 1976	300±1	446±150	50±14	48±6	134±36
Jan. 5, 1977	590±40	30±5	128±4	12±4	24±2
Apr. 27, 1977	380±50	355±100	54±22	226	100±22

[a]Average of duplicate analyses ± ½ the range.

TABLE 15. PCP RELEASE FROM CONTAMINATED LEAF LITTER

Sample	Water			Leaf Residue	Total
	Day 1	Day 2	Day 3		
	mg PCP/liter			mg PCP/kg	
1	0.9	0.3	0.3	4.9	6.2
2	0.6	0.3	0.2	3.9	5.0
3	0.3	0.8	1.1	5.9	8.1
Average	0.6	0.5	0.5	4.8	6.4±1
Original leaf litter sample					6.4±0.3

SOLUBILITY OF PCP IN WATER

The solubility of PCP in unbuffered, distilled water was found to be in the range of 10 to 14 mg/l (ppm) at 23°C with a resulting pH of 5.1. These values are similar to those (14-19 mg/l in water) reported by Bevenue and Beckman (1967). Curves representing the titration of a solution of 9.2 ppm PCP in distilled water with 0.09 M HCl along with the back titration of the acidified solution with 0.1 M NaOH are shown in Figure 3. Concentrations of the phenate and phenol forms calculated for various pH values are given in TABLE 16 along with the pKa values which were found to be an average of 4.5.

The fact that the curves in Figure 3 are not congruent indicates that a nonreversible process occurred upond acidification of PCP and that the solubility of PCP was suppressed. This nonreversible process could result from difficulty in dissolving and dissociating the solid phenol before equilibrium could be attained.

TABLE 16. PCP DISSOCIATION IN WATER AT VARIOUS pH LEVELS

A^- [a]	HA	pH	pKa
0.77	1.08	5.06	5.17
0.716	1.134	4.61	4.80
0.605	1.245	4.32	4.63
0.514	1.34	4.41	4.30
0.444	1.41	3.40	4.50
0.390	1.46	3.39	4.46

[a]Phenate concentration (dissociated form).

[b]Phenol concentration (undissociated form).

[c]pKa calculated from equation (1), p. 14.

Ultraviolet scans of PCP and SPCP in buffer solutions are presented in Figure 4 and summarized in TABLE 17. The absorbance maxima for SPCP agree with the maxima (218.5, 248, and 319 nm) presented in Sadtler (1972), although conditions of analysis, such as pH or ionic strength, were not indicated in Sadtler. However, the molar absorptivities calculated from Sadtler (218.5 nm, 1.3×10^6; 248 nm, 4.4×10^4; 319 nm, 2.7×10^4) are much higher than those in TABLE 1. Without a description of conditions under which the Sadtler data were obtained, it is not possible to speculate on the causes of these differences. The molar absorptivity of pentachlorophenate ion at 319 nm agrees well with that reported above from the data for distilled water.

Absorbance was linear with concentration in the mg/l range at 320 nm for SPCP in 0.1 M borate buffer at pH 10.1 and in 0.1 M phosphate buffer at pH 6.6.

For PCP, absorbance was also linear at pH 2.2 at 2.4 nm in 0.1 M phosphate buffer.

A preliminary study indicated that solubility, as determined by adding prepared buffer to known amounts of PCP, was complete within one day. All subsequent samples were shaken overnight before centrifugation and analysis.

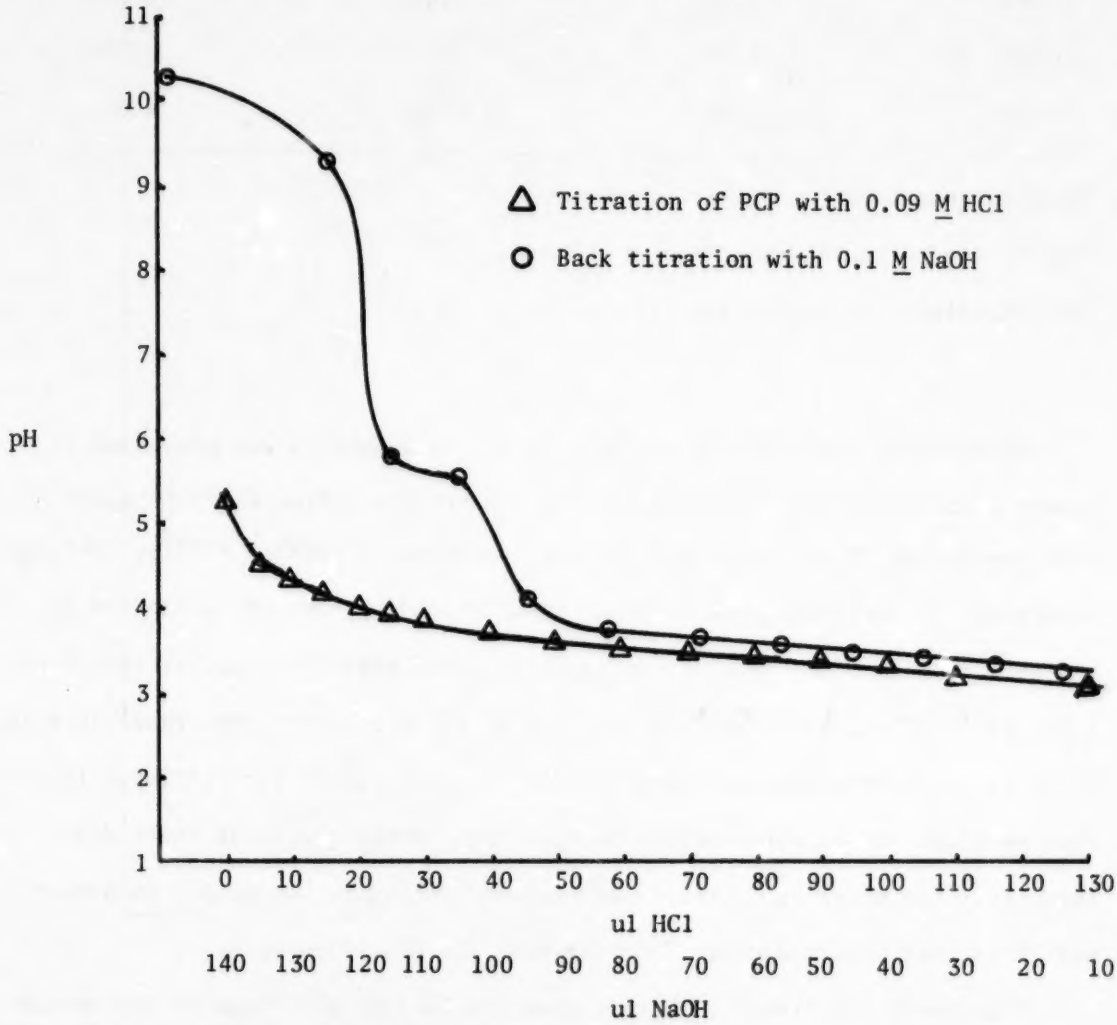


Figure 3. Titration of PCP in aqueous solution.

All samples analyzed by gas chromatography were prepared by adding buffer solutions to known quantities of crystalline PCP. This method was plagued by PCP crystals floating on the surface tension of the solution. Transfer of floating PCP during sample preparation of sample aliquots for extraction may account for at least part of the great variability (i.e., large standard deviations) in solubilities determined by this method (TABLE 18).

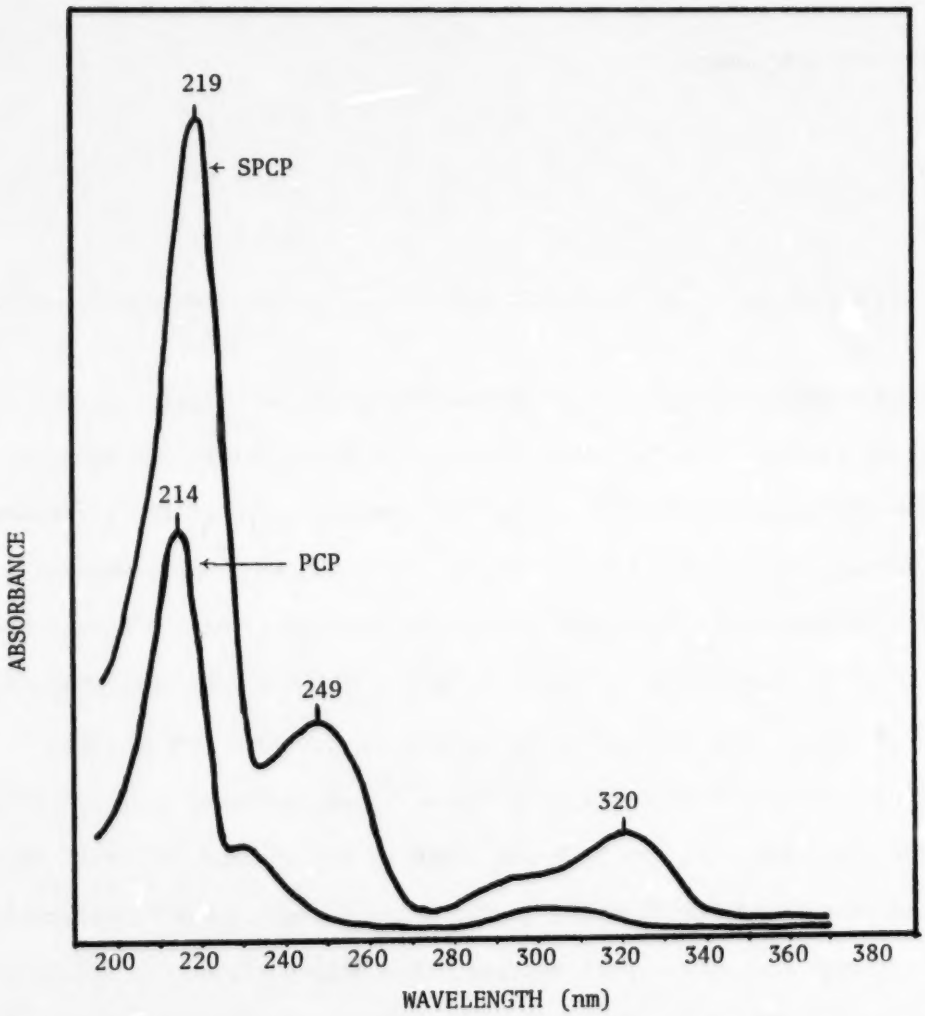


Figure 4. Ultraviolet spectra of pentachlorophenol (PCP) and sodium-pentachlorophenate (SPCP) in 0.1 M buffer.

TABLE 17. SPECTROPHOTOMETRIC CHARACTERISTICS IN THE ULTRAVIOLET OF PENTACHLOROPHENOL AND SODIUM PENTACHLOROPHENATE IN 0.1 M BUFFER

λ max (nm)	Molar Absorptivity
Pentachlorophenol	
214	7.5×10^4
229 (shoulder)	1.7×10^4
Sodium Pentachlorophenate	
219	4.6×10^4
249	1.0×10^4
320	5.0×10^3

Another factor contributing to this variability could be dilution errors, especially in light of the fact that samples had to be diluted as much as 250,000-500,000 times to get them within the working range of the instrument.

At the dilutions needed to use the gas chromatograph, there were no evidences of contaminants in either the hexane-I fraction or the hexane-II fraction prior to methylation. Blanks at pH 2.2 and 6.6 were less than 10 ug/1, and at pH 10.1 less than 200 µg/1. Recoveries ranged from 80% to 130%.

Samples analyzed by absorption in the u.v. were prepared by dissolving PCP in base and acidifying the SPCP solutions to the desired pH. With this method, the problem of PCP floating on the surface tension was considerably reduced, although not entirely eliminated. The higher values for solubilities at pH 2.2 and 6.6 (TABLE 18) for spectrophotometry compared with gas chromatography may indicate that true saturation had not been achieved in samples

analyzed by gas chromatography. Another possibility is that the high dilution ratios needed for samples prepared for gas chromatography consistently produced lower concentrations than samples analyzed spectrophotometrically; for example, pH 10.1 samples were diluted at most only 1000 times before spectrophometric analysis. Or, the difference may indicate that samples analyzed spectrophotometrically were supersaturated, although this possiblity seems remote since there were copious amounts of PCP and SPCP precipitated in the pH 2.2 and 6.6 sample flasks. A further check on this question is needed. In the case of pH 10.1, reproducibility within individual experiments was fairly good, but reproducibility between runs was poor. Apparently, PCP at high pH's readily forms SPCP by reacting with the NaOH in the buffer. Thus, a crude titration was effected by adding PCP to a 0.1 M NaOH-H_3BO_3 buffer. Evidences of this titration effect were found in the marked drops in pH, to as low as 8.5, in solutions of 0.1 M basic buffer to which PCP had been added in what was thought initially to be excess. As a rough estimate from data developed in this study, it seems possible that the solubility of SPCP at pH 10.1

TABLE 18. SOLUBILITY AT 26 C OF PENTACHLOROPHENOL AND SODIUM PENTACHLOROPHENATE AT THREE pH VALUES USING TWO ANALYTICAL METHODS

pH	mg PCP/liter[a]	
	Determination by Gas Chromatography	Determination by Spectrophotometry
2.2	3.0±2.0	7.2
6.6	550 ±240	660
10.1	17,400 ±800	11.900 ±900

[a] ± one standard deviation.

exceeds 0.05 M. A more valid estimate could be obtained using 1 M buffer; however, such a concentrated buffer is outside the realm of what can be found in all but the most extraordinary aquatic environments. Thus, it is perhaps best, albeit vague, to say that SPCP is freely soluble at pH 10.1.

The pKa of PCP is variously reported to be 4.8 (Blackman et al., 1955), 4.82 (Robinson and Bates, 1966), 5.2 (Dyer, 1959), 5.26 (Tiessens, 1929), and 5.3 (Griffith et al., 1938) and 4.5 (above) for distilled water. Even allowing for the variation in reported pKa values, all PCP in solution at pH 2.5 or less is present in the acidic, non-dissociated form. Taking the solubility of PCP at pH 2.2 to be 5.3 mg/1 (TABLE 18), one can calculate a Ksp for PCP ranging from 1.0×10^{-10} (for pKa = 5.3) to 6.3×10^{-10} (for pKa = 4.5).

ACCUMULATION AND ELIMINATION OF PCP IN FISH

These results have been reported previously by Pruitt et al. (1977), and are summarized here.

The 96-hour LC-50 value for the PCP Standard was 0.26 ± 0.01 mg/1 and that for the SPCP Standard was 0.33 ± 0.01 mg/1 (TABLE 19). These values agree with those reported for various aquatic organisms by Goodnight (1942), Holmbert et al. (1972), and Cardwell et al. (1976). The pH of the test solutions ranged from 7.2 to 7.7, indicating that the active form of either standard was the phenate anion.

Exposure of six-month old bluegill sunfish (Lepomis macrochirus) to a sublethal concentration of PCP (0.1 mg/1) resulted in the accumulation of PCP in various tissues. The liver was found to concentrate the largest amount (35,000 ng/g) followed by the digestive tract (21,000 ng/g), gills (6,000 ng/g), and

TABLE 19. LC-50 OF PCP TO LEPOMIS MACROCHIRUS.

Time (hours)	LC-50[a] PCP	SPCP
	mg/liter	
24	0.42 ± .02	0.33
48	0.32 ± .02	0.32 ± .02
72	0.28 ± .03	0.35 ± .01
96	0.26 ± 0.1	0.33 ± .01

[a]Average of three replicate samples ± one-half the range.

muscle (1,000 ng/g) (TABLE 20). Pentachlorophenol was rapidly accumulated by all tissues with equilibrium apparently being established by day 8 (Figure 5).

The efficiency of the extraction procedure was determined to be 93 percent with a standard deviation of 6 percent by the addition of standard PCP to fish

TABLE 20. ACCUMULATION OF PCP IN FISH TISSUE AFTER EXPOSURE TO 0.1 mg PCP/1 WATER.

Tissue	Days Exposure[a] 4	8	16
	µg PCP/g wet weight tissue		
Muscle	0.5±0.1	1.3±0.2	0.4±0.2
Gills	2.6±0.5	6 ±2	5 ±3
Digestive Tract	9 ±2	21 ±4	13 ±5
Liver	35 ±20	25 ±20	23 ±13

[a]Average of triplicate samples ± standard deviation.

tissue and subjecting these "spiked" tissues to the extraction procedures in six replicate analyses. Reagent blanks were obtained by subjecting control fish from PCP-free water to the above extraction and analysis procedure and the PCP content was less than 0.01 ppm in all control samples.

The elimination of PCP from muscle and combined gills, liver, and intestinal tract from Group I fish (4 days exposure) is shown in Figure 6. Similar data for fish from Group II (16 days exposure) are shown in Figure 7. The elimination of PCP from the individual organ tissues from Group II fish is shown in TABLE 21.

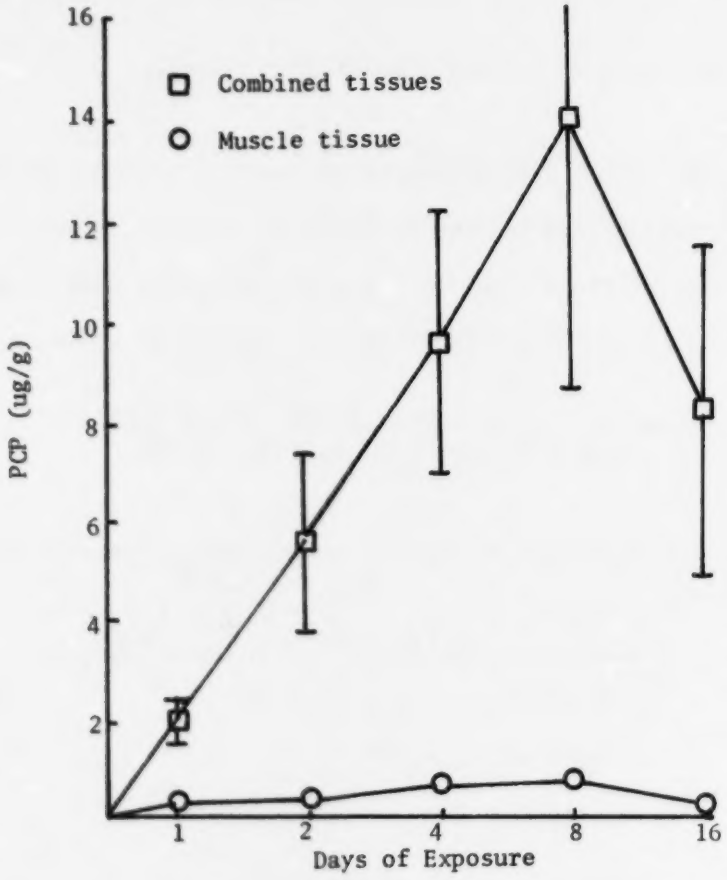


Figure 5. Accumulation of PCP in tissue during exposure to 0.1 ppm PCP. Average of six replicate samples ± standard deviation.

A comparison of Group I with Group II elimination data (Figures 6 and 7) reveals a lag period of 4 days in Group I that was not observed in Group II, indicating that it took the fish about 8 days to develop a mechanisms for PCP elimination. This supports the results of the accumulation study which showed a leveling off and apparent decrease in PCP concentration in tissue after 8 days of continued exposure, possibly representing the time necessary for the fish to develop an enzyme system capable of eliminating PCP. Kobayashi and

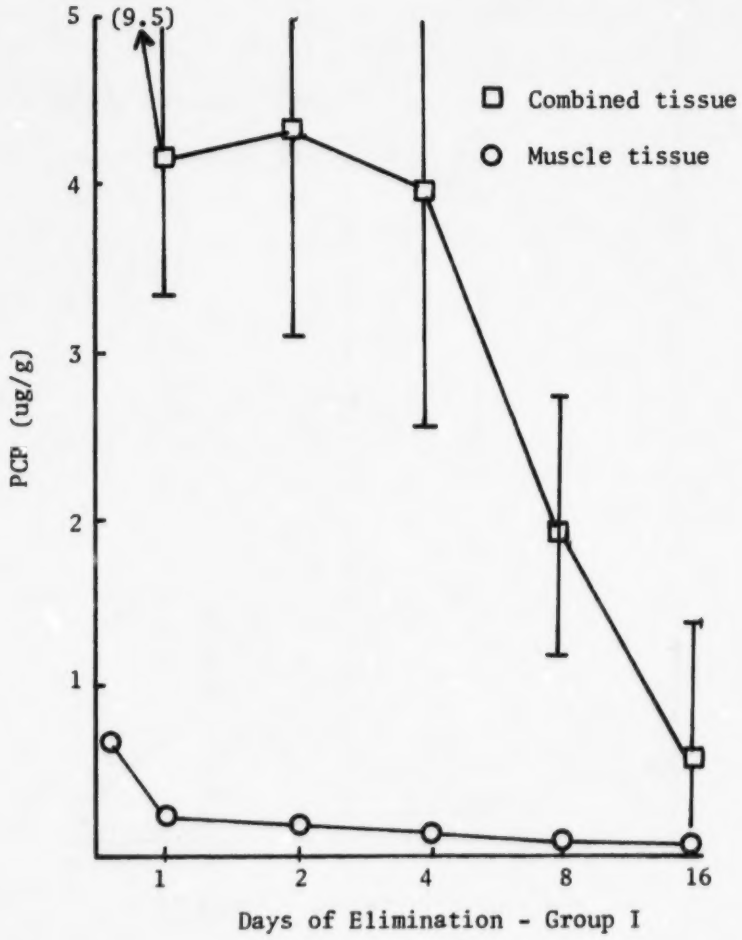


Figure 6. Elimination of PCP from Group I (after 4 days exposure). Average of six replicate samples ± standard deviation.

Akitake (1975) reported that loss of PCP in goldfish (Carassius auratus) was caused by the metabolic transformation of PCP to pentachlorophenylsulfate. This was identical to the conjugate form found in the shortnecked clam by Kobayashi et al. (1970). It has also been reported that fish dispose of such lipid-soluble substances by passive diffusion through the gills (Brodie et al., 1962).

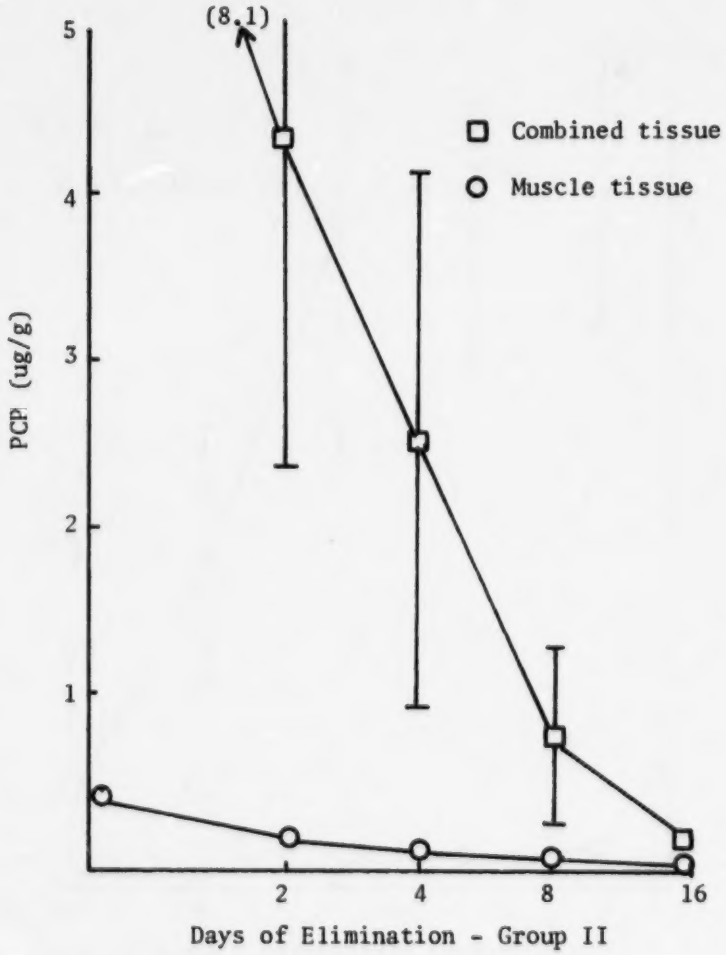


Figure 7. Elimination of PCP from Group II (after 16 days exposure). Average of six replicate samples ± standard deviation.

TABLE 21. ELIMINATION OF PCP FROM GROUP II FISH, PLACED INTO A CLEAN ENVIRONMENT AFTER SIXTEEN DAYS EXPOSURE TO PCP-CONTAMINATED WATER.

Tissue	Days Exposure[a]		
	4	8	16
	µg PCP/g wet weight tissue		
Muscle	0.06±0.02	0.03±0.01	0.03±0.01
Gills	0.16±0.07	0.13±0.09	0.08±0.03
Digestive Tract	2 ±0.9	0.3 ±0.1	0.13±0.02
Liver	0.7 ±0.5	4 ±5	0.6 ±0.5

[a]Average of triplicate samples ± standard deviation.

After 16 days of elimination in PCP-free water, the fish still contained PCP residues (TABLE 21). The liver contained the greatest amount (600 ng/g) followed by the digestive tract (130 ng/g), gills (80 ng/g), and muscle (30 ng/g). By the end of the 16-day elimination study, both Group I fish and Group II fish had eliminated PCP to about the same level (Figures 6 and 7). Thus, after the initial 8-day lag period, the fish were able to eliminate PCP to the same degree regardless of exposure time, yet a longer period would be required for complete depuration.

FOOD CHAIN STUDY

Benthic Organisms

Station A, nearest the spill area, was represented by a slow stream environment with a bottom chracterized by gravel, coarse sand, and sediment. However, stations B, C, and D were lentic habitats characterized by soft, silty, clay substrates. Benthic invertebrates at all four stations were dominated by

the larvae of the two insect families, Chironomidae and Culicidae, and Oligochaeta (TABLE 22 and TABLE 23).

Chironomid larvae appeared as the dominant benthic organisms within intermediate depths of the lake (stations B and D). The dominant chironomid collected was the genus Chironomus. Other genera present were Harnischia, Coelotanypus, and Tanypus. Chironomids were least abundant both near the spill area and in the deepest area of the lake. Undesirable sediment at station A in August and low oxygen levels produced by thermal stratification during August and April at station C may have affected chironomid abundance.

Culicidae, exclusively Chaoborus, present in consistent numbers within the intermediate depths of the lake but were most abundant at the deepest station, C. Due to its tolerance to low levels of oxygen, Chaoborus was the only benthic invertebrate collected at station C during August. Numerically, Chaoborus larvae were dominant at station C throughout the study. Oligochates, primarily tubeficidae, were common only at station B in the upper region of the lake, nearest the head waters.

Diversity of the benthos appeared low throughout the lake. Little seasonal change in the composition of the benthos within each station was observed.

Following the pentachlorophenol spill occurring in December 1976, no organisms were observed in the sample collected from station A nearest the spill area. Absence of organisms may be attributed to the dry conditions preceding the sampling period. Although an unusually large number of chironomid head capsules were collected at station B, its benthic composition following the spill did not appear adversely altered. Subsequent collections in April 1977 did not reflect a numerical decrease among the dominant invertebrates present.

TABLE 22. BENTHIC ORGANISMS COLLECTED DURING AUGUST AND OCTOBER 1976.[a]

Organisms	Stations A[b]	B	C	D
		August		
Diptera				
Chironomidae				
L[c]	15	290		204
P[d]		5		6
Culicidae				
Chaoborus L		87	787	90
Ceratopogonidae L		1		1
Oligocheata	30	87		2
Nematoda		1		
Gastropoda	1			
		October		
Chironomidae				
L		62	8	215
P				3
Chaoborus				
L		222	1350	359
P			12	1
Oligocheata		72		
Nematoda		1		11
Copepoda			2	

[a]Total number of organisms from three (15.5 cm x 15.5 cm) Eckman dredge samples.

[b]Stream was dry in October.

[c]Larvae.

[d]Pupae.

TABLE 23. BENTHIC ORGANISMS COLLECTED DURING JANUARY AND APRIL 1977.[a]

Organisms	Stations A[b]	B	C	D
		January		
Chironomidae				
L[c]		65	56	158
P[d]			1	
Ceratopogonidae L		1		1
Cuclicidae		170	3024	286
Chaoborus L				
Oligocheata		132		1
		April		
Chironomidae				
L		379		271
P		2		1
Chaoborus				
L		15	298	238
P		2	6	15
Ceratopogonidae L		1	1	3
Dytiscidae L		1		
Nematoda		1	2	4
Oligocheata		4		1

[a] Total number of organisms from three (15.5 cm x 15.5 cm) Eckman dredge samples.

[b] No invertegrates were observed at site A.

[c] Larvae.

[d] Pupae.

Food Habits

Results indicate that small sunfish (Lepomis spp.) and intermediate sized bluegill (L. macrochirus) fed predominantly on small bottom organisms, primarily chironomid larvae (TABLE 24). Benthic oraganisms together consitute 77.3% of the diet of the smaller sunfish (<50 mm) collected. Larger sunfish also appeared to utilize benthic prey, largely in the form of chironomid larvae. In addition to insect larvae, larger warmouth (L. gulosus) also utilized smaller fish as food.

Larger game fish, collected with the aid of a gill net were observed to feed on fewer numbers of larger food items (TABLE 25). Crayfish constituted the bulk of the observed channel catfish (Ictalurus punctatus) ration, however, large numbers of chironomid larvae and the presence of a smaller fish within the stomachs suggests a diverse diet. The black bullhead (I. melas) and yellow bullhead (I. natalis) appeared to feed on a wide range of food items indicated by both insect larvae, vegetation, and fish scales found in the gut.

Rough fish, golden shiner (Notemigonus crysoleucas) and sharpfin chub-sucker (Erimyzon tenuis), appeared to be grazers with their respective rations consisting primarily of filamentous algae and microcrustaceans (TABLE 26).

Among the three dominant benthic groups, chironomid larvae, Chaoborus larvae, and Oligochaetes, chironomids appeared as the most utilized food source by fishes. Although chironomid larvae were represented in the stomachs of most fish species, they comprised the dominant food source of small and intermediate length sunfish (Lepomis spp.). Larger sunfish, particularly warmouth (L. gulosus) and green sunfish (L. cyanellus), also appeared to prey on smaller fish (Calhoun, 1966). Although no largemouth bass (Micropterus salmoides) were collected with identifiable stomach contents, Snow (1971) and Bennett (1962)

TABLE 24. STOMACH CONTENTS OF SUNFISH COLLECTED FROM AUGUST 1976 THROUGH APRIL 1977.

Species	Lepomis Spp.		Lepomis macrochirus		Lepomis machrochirus	Lepomis cyanellus		Lepomis gulosus	
Length range (SL in mm)	0-50		51-100		107	51-100		101-150	
No. of stomachs	30		6		1	3		5	
Food Items									
Insecta									
Diptera									
Chironomidae-L[c]	658[a]	90.3[b]	349	83.3	138	62	100.0		
-P[d]	17	38.7	22	50.0		35	100.0	4	20.0
Culicidae-L	4	25.8	6	50.0	48				
-P									
Ceratopogonidae-L	4	3.2	10	16.7	26	1	33.3		
Ephemeroptera-L									
Baetidae-L			1	16.7					
Trichoptera-L						7	100.0		
Pelecypoda	2	6.5							
Crustacea									
Copepoda	267	77.4	27	83.3	7	3	66.7		
Ostracoda	37	54.8	13	33.3	1				
Cladocera	1111	22.6	10	16.7	1	1	33.3		
Nematoda	22	22.6	1	16.7					
Hydracarina	1	3.2	2	16.7					
Algae									
Flamentous		6.5							
Miscellaneous Vegetation		25.8							
Teleost									
Fish scales		25.8							

[a] Total number of organisms.

[b] Frequency of occurrence.

[c] Larvae.

[d] Pupae.

TABLE 25. STOMACH CONTENTS OF CATFISH COLLECTED FROM AUGUST 1976 THROUGH APRIL 1977.

Species	Ictalurus melas		Ictalurus natalis	Ictalurus punctatus	
Length range (SL in mm)	161-260		180	351-410	
No. of stomachs	4		1	4	
Food items					
Insecta					
Diptera					
Chironomidae-L[c]	1[a]	25.0[b]	3	202	25.0
-P[d]			1	173	25.0
Culicidae -L	23	50.0	4	2	25.0
-P	1	25.0			
Ephemeroptera-L			2		
Baetidae -L					
Crustacea					
Copepoda					
Cladocera					
Decapoda				3	75.0
Asticadie					
Teleost	1	25.0	14	1	25.0
Fish scales	8	50.0			
Miscellaneous Vegetation		25.0			

[a]Total number of organisms

[b]Frequency of occurence

[c]Larvae

TABLE 26. STOMACH CONTENT OF NON-GAME FISH COLLECTED FROM AUGUST 1976 THROUGH APRIL 1977.

Species	Erimyzon tenuis	Erimyzon tenuis		Erimyzon tenuis		Notemigonus chysoluecas	
Length range (SL in mm)	101-150	151-200		201-250		101-151	
No. of stomachs		2		6		9	
Food items							
Insecta							
Diptera							
Chironomidae-L[c]	3[a]	92[a]	100.0[b]	49	100.0	109	44.4
-P[d]		1	50.0	7	16.7		
Culicidae -L		92	50.0	27	33.3		
-P		1	50.0				
Ceratopogonidae-L		5	50.00	4	33.3		
Miscelaneous-L				3	50.0	1	11.1
Trichoptera-L		4	50.0	14	50.0		
Crustacea							
Copepoda	5	93	100.0	971	100.0	8	33.3
Ostracoda		85	100.0	427	100.0	19	22.2
Cladocera	193	2417	100.0	17357	83.3	153	77.8
Rotifera						116	11.1
Nematoda				2	33.3		
Oligochaeta				2	16.7		
Hydracarina				1	16.7		
Algae							22.2
Unicellular							55.6
Flamentous							

[a]Total number of organisms

[b]Frequency of occurrence

[c]Larvae

[d]Pupae

contend that fish and larger invertebrates constitute the major portion of their diet.

The black bullhead (Ictalurus melas), yellow bullhead (I. natalis) and channel catfish (I. punctatus) appeared as omnivorous feeders in which much of their ration was also derived either directly or indirectly from the benthos. Bullheads appeared to feed on both aquatic dipteran larvae and smaller fishes with aquatic vegetation also utilized by black bullhead. Crayfish appeared to constitute the bulk of the channel catfish ration with chrionomid larvae and smaller fish also present.

Both non-game species collected, golden shiner (Notemigonous crysoleucas) and sharpfin chubsucker (Erimyzon tenuis) appeared as discriminant grazers upon non-benthic bood items. Filamentous algae appeared to constitute the major component of the larger golden shiner ration with chironomid larvae representing a minor portion. Sharpfin chubsucker appeared to graze primarily upon microcrustaceans with chironomid larvae also constituting a secondary importance.

In general it appears that the basis of the food chain within Country Club Estates Lake is channeled through the benthic invertebrate community, particulary in regard to game fish species. The benthic food source is of immediate significance to the younger components of both the sunfish and catfish populations (Calhoun, 1966). Smaller sunfish in turn furnish forage to larger predaceous game species (i.e., larger sunfish, largemouth bass, catfish). Non-game species appear less dependent upond a benthic food source, utilizing prey items suspended in the water column.

REFERENCES

1. American Public Health Association. 1971. Standard Methods for the Examination of Water and Waste-water. 13th ed. American Public Health Association, Inc., New York. p. 562-576.

2. Arsenault, R. D. Pentachlorophenol and Contained Chlorinated Dibenzodioxins in the Environment. Proc. Amer. Wood Preser. Assoc. Annual Meeting, Atlanta, Ga., April, 1976, pp. 1-25.

3. Bennett, G. W. Management of Artifial Lakes and Ponds. Reinhold Publishing Corp., New York, 1972, 283 pp.

4. Bevenue, A., and H. Beckman. Pentachlorophenol: A Discussion of Its Properties and Its Occurrence as a Residue in Human and Animal Tissues. Residue Rev., 19: 83-134, 1967.

5. Blackman, G. E., M. H. Parker, and G. Garton. The Physiological Activity of Substituted Phenols. II. Relations Between Physical Properties and Physiological Activity. Arch. Biochem. Biophys. 54, 55-71, 1955.

6. Brodie, B. B. and R. P. Maickel. Mode of Action of Drugs. Proc. Inst. Intern. Parmacol. Meeting, 1962, pp. 299-324.

7. Buhler, D. R., M. E. Rasmusson, and H. S. Nakave. Occurrence of Hexachlorophene and Pentachlorophenol in Sewage and Water. Env. Sci. Tech., 7, 1973. pp 929-934.

8. Buser, H. R. Analysis of Polychlorinated dibenzo-p-dioxins and Dibenzofurans in Chlorinated Phenols by Mass Fragmentography. J. Chromatogr. 107, 1975. pp 295-310.

9. Buser, H. R. High-Resolution Gas Chromatography of Polychlorinated Dibenzo-p-dioxins and Dibenzofurans. Anal. Chem., 48, 1976. pp 1553-1557.

10. Calhoun, A. Inland Fisheries Management. Calif. Dept. of Fish and Game., 1966. pp 546.

11. Cardwell, R. D., D. G. Foreman, T. R. Payne, and D. J. Wilbur. Acute Toxicity of Selected Toxicants to Six Species of Fish. U.S. EPA-600/3-76-008, 1976. pp 1-117.

12. Cote, R. P. A Literature Review of the Toxicity of Pentachlorophenol and Pentachlorophenates. Environmental Protection Service Manuscript Report, 72-2, Halifax, N.S., 1972. pp 1-14.

13. Crosby, D. G. Photodegradation of Pesticides in Water. Amer. Chem. Soc., Advances in Chemistry Ser., 111, 1972. pp 173-188.

14. Crosby, D. G., K. W. Moilanen, and A. S. Wong. Environmental Generation and Degradation of Dibenzodioxins and Dibenzofurans. Env. Health Perspect., 5, 1973. pp. 259-266.

15. Crosby, D. G., and A. S. Wong. Photochemical Generation of Chlorinated Dioxins. Chemosphere, 5, 1976. pp. 327-332.

16. Crummet, W. B. and R. H. Stehl. Determination of Chlorinated Dibenzo-p-dioxins and Dibenzofurans in Various Materials. Env. Health Perspect., Issue #5, HEW, Pub. #NIH 74-218, 1973. pp. 15-25.

17. Cserjesi, A. J., and E. L. Johnson. Methylation of Pentachlorophenol by Trichoderma virgatum. Canadian J. Microbiol., 18, 1972. pp. 45-49.

17(a) Dougherty, R.C., and K. Pitrowska. Screening by Negative Chemical Ionization Mass Spectrometry for Environmental Contamination with Toxic Residues. Proc. Natl. Acad. Sci. 73(6): 1777-1781, 1976.

18. Dyer, D. L. The Effect of pH on Solubilization of Weak Acids and Bases. J. Colloid Sci., 14, 1959. pp. 640-645.

19. Goodnight, C. J. Toxicity of Solium Pentachlorophenate and Pentachlorophenol to Fish. Ind. Eng. Chem., 34, 1942. pp. 868.

20. Griffith, R. O., A. McKeown, and W. J. Shutt. Annual Tables of Constants and Numerical Data, No. 22, Vol. 13. Herman, Paris, 1938.

21. Holmbert, B.,. Jensen, A. Larsson, K. Lewander, and M. Olsson. Metabolic Effects of Pentachlorophenol (Penta) on the Eel Anguilla anguilla L. Comp. Biochem. Physiol. 43(B), 1972. pp. 171-183.

22. Kirsch, E. J. and J. E. Etzel. Microbial Decomposition of PCP. Journal WCPF, Vol. 45, No. 2, 1973. pp. 359-364.

23. Kobayashi, K., H. Akitake, and T. Tomiyama. Studies on the Metabolism of Pentachlorophenate, A Herbicide, In Aquatic Organisms-II. Biochemical Change of PCP in Sea Water by Detoxification Mechanisms of Tapes philippinarum. Bull. Jap. Soc. Sci. Fish, 36, 1970. pp. 96-102.

24. Kobayashi, K. and H. Akitake. Studies on the Metabolism of Chlorophenols in Fish--III. Isolation and Identification of a Conjugated PCP excreted by Goldfish. Bull. Jap. Soc. Sci. Fish., 41, 1975. pp 321-327.

25. Kobayashi, K. S. Kimura, and H. Akitake. Studies on the Metabolism of Chlorophenols in Fish--VII, Sulfate Conjugation of Phenol and PCP by Fish Livers. Bull. Jap. Soc. Sci. Fish., 42, 1976. pp. 171-177.

26. Mississippi Air and Water Pollution Control Commission, Court Hearing, Jackson, Mississippi, January 14, 1975.

27. Munakata, K. and M. Kuwahara. Photochemical Degradation Products of Pentachlorophenol. Residue Rev., 25, 1969. pp. 13-23.

28. Pierce, R. H., Jr., C. R. Brent, H. P. Williams, and S. G. Reeves. Pentachlorophenol distribution in a Fresh-water Ecosystem. Bull. Env. Contam. Toxicol., 18(2): 251-257, 1977.

29. Plimmer, J. R. Technical Pentachlorophenol: Origin and Analysis of Base-insoluble contaminants. Env. Health Perspect., U.S. Dept. of Ag., 1973. pp. 41-47.

30. Pruitt, G. W., B. J. Grantham, and R. H. Pierce, Jr. Accumulation and Elimination of Pentachlorophenol in the Bluegill, Lepomis macrochirus, Trans. Amer. Fish. Soc., 1977. In press.

31. Rappe, C., and C. A. Nilsson. An Artifact in the Gas Chromatographic Determination of Impurities in Pentachlorophenol. J. Chromatogr. 67, 1972. pp. 247-253.

32. Robinson, R. A., and R. G. Bates. Dissociation of Some Substituted Phenols in 50% Aqueous Methyl Alcohol. J. Res. Nat. Bur. Stand. A 70, 1966. pp. 553-556.

33. Rudling, L. Determination of PCP in Organic Tissues and Water. Water Research, 4, 1970. pp. 533-537.

34. Sadtler Research Laboratories, Inc. The Sadtler Standard Spectra, UV-112, Vol. 1, 1970.

35. Schlenk, H., and J. Gellerman. Esterification of Fatty Acids with Diazomethane on a Small Scale. Anal. Chem., 32, 1960. pp. 1412-1414.

36. Shafik, T. M. Determination of PCP and Hexachlorophene in Human Adipose Tissue. Bull. Environ. Contam. Toxicol, 10, 1973. pp. 57-63.

37. Snow, H. Harvest and Feeding Habits of Largemouth in Murphy Flowage, Wisconsin. Wisc. Dept. Nat. Res. Tech. Bull. No. 50, 1971. pp. 25.

38. Stark, A. Analysis of Pentachlorophenol in Soil, Water, and Fish. J. Agric. Food Chem., 17: 871-873. 1969.

39. Tiessens, G. J. Trichloro- and the Higher Chlorophenols and Their Electrical Conductivity in Water. Rec. Trav. Chem., 48, 1929. pp. 1066-1068.

40. U. S. Environmental Protection Agency. Manual of Analytical Methods for the Analysis of Pesticide Residues in Human and Environmental Samples, Chapter 5, U.S. EPA, Env. Toxicol. Div., Research. Triangle Park, North Carolina, 1974.

41. Zitko, V., O. Hutzinger, and P.M.K. Choi. Determination of Pentachlorophenol and Chlorobiphenyls in Biological Samples. Bull. Env. Contam. Toxicol., 12, 1974. pp. 649-653.

TABLE A-1 PHYSICAL PARAMETERS FROM WATER SAMPLING SITES FROM FEBRUARY 1975 TO MAY 1976

Date	Station	Temp ^{o}C	pH	D.O. mg/1	TOC mg/1
2-27-75	1	15		8.8	12.6
	2	16		8.4	14.2
	3	15		9.6	12.4
	4	16		9.4	NA[a]
	5	16		8.6	14.0
	6	16		9.4	17.6
4-24-75	1	19		6.4	6.3
	2	19		6.0	6.2
	3	22		9.8	7.9
	4	22		9.5	7.9
	5	22		9.5	7.9
	6	22		9.3	7.4
6-28-75	1	23		0.6	NA
	2	23		4.3	20.9
	3	30		9.0	17.5
	4	30		9.8	17.4
	5	30		9.6	NA
	6	30		9.4	16.2
8-2-75	1	NA		NA	NA
	2	NA		NA	NA
	3	28	6.2	7.8	16.9
	4	29	6.3	6.6	18.1
	5	29	6.3	7.2	17.2
	6	29	5.8	6.6	15.5

(continued)

TABLE A-1. (CONTINUED)

Date	Station	Temp ^{o}C	pH	D.O. mg/l	TOC mg/l
10-11-75	1	20	5.1	8.0	24.9
	2	20	5.2	6.0	24.5
	3	22	NA	4.0	18.5
	4	23	NA	8.0	17.2
	5	24	6.2	9.0	19.6
	6	24	5.0	8.8	17.7
12-6-75	1	12	4.8	5.9	24.1
	2	10	4.6	6.6	NA
	3	15	5.1	10.2	16.6
	4	15	6.5	10.4	16.7
	5	15	6.6	10.6	16.9
	6				
2-7-76	1	14	6.0	8.2	20.0
	2	NA	NA	NA	NA
	3	11	6.4	8.9	15.8
	4	12	6.5	9.7	19.8
	5	11	6.5	9.7	19.8
	6	11	6.3	10.0	22.2
5-3-76	1	18	6.2	5.9	21.7
	2	NA	NA	NA	NA
	3	22	6.8	8.0	15.6
	4	NA	NA	NA	NA
	5	23	6.7	8.7	15.4
	6	22	6.6	8.2	17.9

[a]Not analyzed.

TABLE A-2 PHYSICAL PARAMETERS FROM WATER SAMPLING SITES FROM AUGUST 1976 - APRIL 1977

Date	Station	(depth) meters	Temp °C	D.O. mg/1	pH
August, 1976	A	0.3	27	N.A.[a]	5.8
	B	0.3	26	8.0	5.7
		2.0	26	7.4	
	C	0.3	26	8.7	6.1
		5.0	20	0.2	
	D	0.3	27	7.5	6.0
		2.0	25	5.0	
October, 1976	A	Dry	N.A.	N.A.	N.A.
	B	0.3	16	10.4	6.6
		1.8	12	10.2	
	C	0.3	15	10.6	6.5
		4.0	12	2.7	
	D	0.3	17	10.5	6.5
		1.5	12	10.6	
January, 1977	A	0.3	12	N.A.	6.8
	B	0.3	12	11.8	6.5
		1.8	7	9.5	
	C	0.3	12	12.2	6.5
		6.0	6	6.2	
	D	0.3	12	12.8	6.5
		2.6	7	6.0	
April, 1977	A	0.3	25	N.A.	6.5
	B	0.3	24	6.5	6.5
		2.0	18	5.0	
	C	0.3	26	6.7	6.6
		5.0	15	0.3	
	D	0.3	24	6.6	6.6
		1.5	20	3.0	

[a] Not analyzed.